clinical environment'; haematologist Dr Archie McKinney; and two cardiologists at Duke University during his training—Clinical Cardiologist Dr William Floyd, and Dr Joseph Greenfield (Chief of Cardiology at the Veteran's Administration Hospital affiliated with Duke) who mentored his research and showed him that 'the highest standards established in the research laboratory could carry over into the clinical setting'.

Today, Dr Elion is a Cardiologist and Clinical Associate Professor of Medicine at Brown University, and part of the Cardiovascular Institute at The Miriam Hospital and its parent organization, Lifespan, with a continued interest in the transition to digital working and use of artificial intelligence in cardiology.

He met his wife Kathleen in 1974 during his CCU rotation when she was a nurse, and they have a son Christopher and daughter Leigh. Trudy Elion never married. She was engaged but her fiancé Leonard Canter became died of bacterial endocarditis in 1941.

'Trudy never talked about Leonard, although she did allude to his existence', he said. 'I have a marvellous collection of correspondence between Trudy and Leonard and a diary she kept. There is a fateful (to me as a Cardiologist) letter from Leonard explaining that he did not get a job due to a heart murmur. That turned out to be caused by the heart infection that killed him'.

As an avid opera fan, Madame Butterfly was her favourite. Her diary and a program from the New Year's Eve performance of 1937 recalls her first kiss with Leonard after seeing the performance together.

'For the rest of her life, she must have thought about that date with Leonard whenever she saw Madame Butterfly', he pondered.

Inspiring young scientists

Trudy Elion—who received the National Medal of Science from President George H. W. Bush, on 16 September 1991—will be remembered for medicines she developed and the scientific approach that produced those drugs which led to the Nobel Prize.

'I think she would want to be remembered as someone who helped to save lives', he added, 'who inspired the young scientists working in her lab. Her legacy will be one of paying attention to the little effects in an experiment; they can be as revealing or more revealing than the big effects'.

Her humility, sense of humour, love of opera, egalitarian approach to human interactions, and the pursuit of science are all hallmarks of what she leaves behind.

He remains proud to have been a nephew to 'Aunt Trudy'—'a wonder woman', he said, 'that helped to form my approach to science, to medicine, and to life'.

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Global spotlights

PCSK9 vaccine: so near, yet so far!

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Passive immunotherapy using monoclonal antibodies (mAbs) inhibiting PCSK9, as a critical negative regulator of LDL receptor (LDLR), has been a breakthrough in the field of lipid-lowering therapy during the past decade. However, long-term clinical use of the mAbs is associated with limitations such as short *in vivo* half-life, which necessitate frequent administration and high cost, some tolerability problems, and possible induction of host anti-mAbs. To circumvent such limitations, research on active immunotherapy and vaccination approaches against PCSK9 has recently been flourished.

The fundamental feature of a PCSK9 vaccine is the capacity to trigger the generation of host anti-PCSK9 antibodies, which can properly neutralize the PCSK9/LDLR interaction. Using a combination of the AFFITOME® strategy and nanoliposome platform technology, we have recently designed a novel antiPCSK9 vaccine formulation, called Liposomal Immunogenic Fused PCSK9-Tetanus peptide plus Alum adjuvant (L-IFPTA).[1] The components of the L-IFPTA formulation have been selected in a way that provide prerequisites for the safe and effective activation of the immune system. The main challenge for designing a safe vaccine against self-antigens, such as PCSK9, is to effectively break down B-cell tolerance, while avoiding activation of the destructive auto-reactive T-cell response. Hence, it is important during vaccine development to exclude peptide antigens that are able to induce specific T-cell responses. However, B cells need aid from $CD4^+$ T helper (Th) cells for efficient activation and differentiation into durable plasma and memory cells. A strategy for inducing sufficient generation of autoantibodies is to physically conjugate a B-cell epitope of

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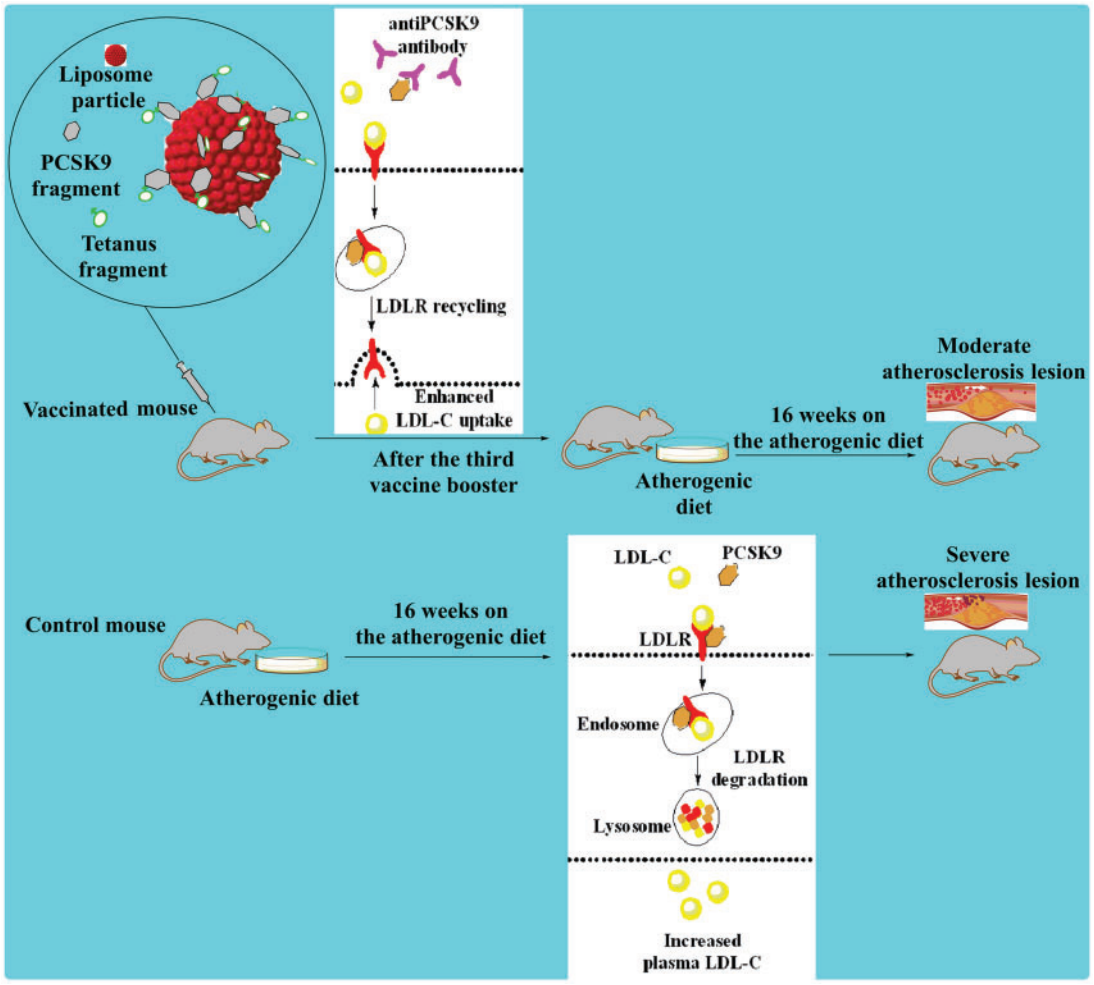

Figure 1 L-IFPTA vaccine in the preventive approach. Mice were immunized with the L-IFPTA vaccine four times in bi-weekly intervals. The L-IFPTA vaccine induced long-term production of specific antiPCSK9 antibodies that could effectively inhibit PCSK9/LDLR interaction, thereby increasing the LDL receptor frequency on the surface of hepatocytes and consequently enhancing LDL-C uptake from the bloodstream. After the last vaccination, mice were fed with an atherogenic diet for up to 16 weeks. At the end of the study, a reduced progression of atherosclerosis lesion was observed in the aortic arch area in vaccinated mice compared with control mice. L-IFPTA, Liposomal Immunogenic Fused PCSK9-Tetanus peptide plus Alum adjuvant; LDLR, LDL receptor.

self-antigen to a foreign Th epitope. To this end, the L-IFPTA vaccine contains two different epitopes belonging to PCSK9 and tetanus toxin proteins. The PCSK9 fragment provides a B-cell epitope, which mimics an N-terminal sequence responsible for PCSK9 bound to LDLR. The amino acid sequence of the PCSK9 fragment was designed using AFFITOME® technology[2,3] in a way that is different from the native sequence, which can be identified as foreign by the immune system and thus overcome the self-tolerance. The PCSK9 fragment has a close similarity between humans and rodents, thereby vaccine-generated antibodies have the potential to block the PCSK9/LDLR interaction in both species. To enhance the $CD4^+$ T-cell response, tetanus peptide as a foreign Th epitope was coupled to the PCSK9 fragment. We indicated that the L-IFPTA vaccine can inhibit PCSK9-specific T-cell activation, while promoting tetanus-specific T-cell response that enhances PCSK9-specific B-cell activation without safety concerns.[1] Another important feature that can highly enhance the peptide immunogenicity to induce effective antibody responses is antigen polyvalency. Polyvalent antigens enable a better cross-linking of the B-cell receptors and robustly induce the proliferation of B cells and can even reverse the anergic phase of self-reactive B cells. Strong B-cell activation is accompanied by elevated expression of molecules that permit successive connections with Th cells, causing the generation of memory and plasma B cells that can persistently produce high-titre antibody responses. Notably, we utilized nanoliposome particles as a carrier adjuvant to provide an antigen polyvalency decoration. Liposome nanoparticles are biodegradable and biocompatible bilayer vesicles that can serve as a multivalent platform for antigen display. We demonstrated that surface-displayed peptide nanoliposomes (L-IFPTA) elicited higher and more durable titres of anti-PCSK9 antibody compared with the peptide alone.[1]

Hitherto, the efficacy of L-IFPTA vaccine has been shown in different animal models.[1,4–10] The L-IFPTA vaccine induced the production of

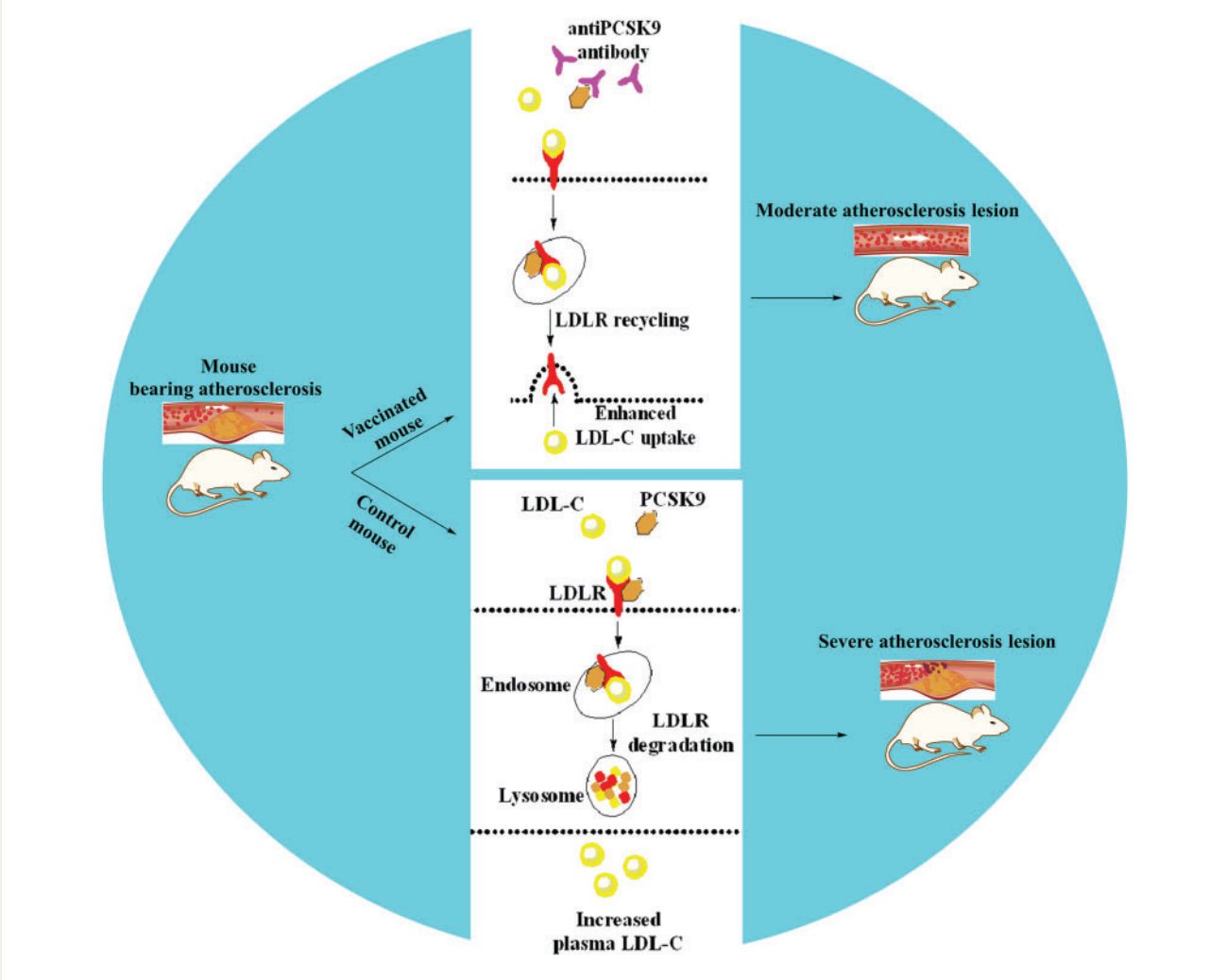

Figure 2 L-IFPTA vaccine in the therapeutic approach. Mice were fed an atherogenic diet for 16 weeks. Hypercholesterolaemic mice bearing atherosclerosis lesions were then immunized with L-IFPTA vaccine four times in bi-weekly intervals. After the immunization period, atherosclerosis lesions with moderate severity were observed in the vaccinated mice, while control mice suffered from severe atherosclerotic lesions. L-IFPTA, Liposomal Immunogenic Fused PCSK9-Tetanus peptide plus Alum adjuvant.

functional anti-PCSK9 antibodies in BALB/c mice, which were both safe and long-lasting (monitored by up to 1 year).[1] Further investigation showed that immunization with the L-IFPTA vaccine could exert long-lasting preventive (*Figure 1*) and therapeutic (*Figure 2*) effects against hypercholesterolaemia and atherosclerosis in mice.[4,5] Notably, a significant negative correlation between anti-PCSK9 antibody titres with serum levels of the free PCSK9 and LDL-C was observed. In a study exploring the therapeutic efficacy of vaccine in C57BL/6 mice with established hypercholesterolaemia and atherosclerosis, four injections of vaccine caused reductions in total cholesterol (TC), LDL-C, and very low-density lipoprotein cholesterol (VLDL-C) by up to 44.7%, 51.7%, and 19.2%, respectively. Long-term follow-up in the same animals indicated a significant reduction of LDL-C by up to 42% in vaccinated hypercholesterolaemic mice over 16 weeks post-prime vaccination. Importantly, the L-IFPTA vaccine could ameliorate the severity of atherosclerosis lesions in hypercholesterolaemic mice; as 'intima to media thickness' and size of lesions were significantly reduced by 46% and 39%, respectively.[4] Similar to the therapeutic effect, we demonstrated that the L-IFPTA vaccine could also exert a preventive impact against hypercholesterolaemia and atheroma formation in mice. Long-term monitoring showed that during 12 weeks after the last vaccination shot, antibody titres in vaccinated mice remained significantly high, which was accompanied with up to −82.5% and −88.14% reductions in serum levels of TC and LDL-C as well as smaller lesion size (−24%) in the aortic arch of vaccinated mice on a severe atherogenic diet.[5] From a mechanistic standpoint, the L-IFPTA vaccine was found to induce the generation of functional antibodies that could specifically target circulating PCSK9 and hinder its interaction with LDLR, which resulted in enhanced expression of LDLR on the surface of hepatocytes and ensuing increase in cholesterol clearance from the bloodstream.

Aside from its inherent atherogenic impact, hypercholesterolaemia can promote systemic and vascular inflammation, which accelerate atherosclerotic lesion formation and instability. We showed that the L-

IFPTA vaccine reduced the elevated levels of interferon (IFN)-γ-producing T cells in hypercholesterolaemic mice. The pro-inflammatory IFN-γ cytokine can induce and worsen atherosclerosis progression *via* inducing lipid accumulation and foam cell formation in the vascular wall and affecting cellular composition of the plaque. In contrast, IL-4 and IL-10 cytokines secreted by Th2 cells counteract the IFN-γ production. A high level of Th2 cells in the circulating blood is independently associated with a decrease in the carotid plaque thickness and a reduced risk of acute myocardial infarction. The L-IFPTA vaccine could significantly increase the levels of Th2 cells and production of IL-4 and IL-10 cytokines in the hypercholesterolaemic mice. The present findings can provide implications regarding the immunological safety of the L-IFPTA^{+} vaccine.

Altogether, the aforementioned findings support the potential of L-IFPTA vaccine as a potent candidate for the management of dyslipidaemia and atherosclerotic cardiovascular disease (CVD). Nevertheless, clinical data with L-IFPTA are still lacking until additional steps are taken. We have just started the preclinical toxicity studies with L-IFPTA to evaluate possible toxic effects of the vaccination on vital organs. In parallel, the immune safety and efficiency of the vaccine in eliciting anti-PCSK9 antibody response are being studied in non-human primates. While the path to achieving an effective PCSK9 vaccine still requires significant work, the extant preclinical evidence on the safety and efficacy of the L-IFPTA formulation is encouraging to hold a promise for a phase I trial in near future.

Conflict of interest: Maciej Banach has the followings to disclose: speakers bureau: Amgen, Herbapol, Kogen, KRKA, Polpharma, Mylan/Viatris, Novartis, Novo-Nordisk, Sanofi-Aventis, Teva, Zentiva; consultant to Abbott Vascular, Amgen, Daichii Sankyo, Esperion, Freia Pharmaceuticals, Novartis, Polfarmex, Sanofi-Aventis; Grants from Amgen, Mylan/Viatris, Sanofi and Valeant. Other authors have nothing to disclose.

Data availability

There is no raw data associated with this article.

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