# Vaccine Research

## High-growth enterovirus 71 strains and vaccines

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Enterovirus 71 (EV71) has caused several epidemics of hand, foot and mouth diseases (HFMD) in Asia and now is also being recognized as an important neurotropic virus. Effective vaccine against EV71 infection are urgently needed.

Current enterovirus 71 (EV71) vaccine candidates evaluated in clinical trials include genotype B2 (Singapore), B4 (Taiwan), and C4 (China) vaccine strains. These vaccine viruses could not grow efficiently in cell cultures (about 107 PFU/ml). The current invention discloses high growth genotype B5 viruses (>108 PFU/ml) that were generated by serial passages and plaque selections in Vero cells which are the most popular cell line for production of human vaccines. Genotype B5 viruses recently caused large scale of EV71 epidemics in Taiwan and South Eastern Asia. The current invention could induce better vaccine production against genotype B5 viruses.

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Patent status: TWI638048, CN108055827A, MYPI2017703328 (Pending)

## Adenoviral vector-based vaccine against enterovirus infection

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Among Enterovirus A, enterovirus 71 (EV71) and coxsackievirus A (CVA) are the most common causative factors of hand, foot, and mouth disease (HFMD) and other neurological disorders. Severe neurological disorders, including encephalitis, acute flaccid paralysis, pulmonary edema, and hemorrhaging, culminating in fatality, particularly in EV71-infected children under 5 years old, have been reported. Because EV71 and CVA infections can potentially become a new threat to global public health, effective antiviral drugs and prophylactic vaccines are urgently needed.

In a previous study, the research team produced a formalin-inactivated EV71 strain E59 (FI-EV71) vaccine candidate formulated with alum adjuvant, and found that FI-EV71 displayed high efficacy in the hSCARB2-Tg mouse challenge model. In a human phase I clinical trial, FI-EV71 was safe and could elicit strong neutralizing antibody responses against current circulating EV71 isolates, but failed to protect against CVA16 infections.

The present invention relates to a novel adenoviral vector-based DNA vaccine for generating immunity against enterovirus infection. In another embodiment, the present invention provides a recombinant adenoviral vector which comprises an expression cassette encoding a 3C protease or a 3CD protease of an enterovirus. The invention discloses that vaccination of the recombinant adenoviral vector as described induces enhanced protective immunity against enterovirus infection, especially cellular (T cell) immune responses. The invention also discloses that vaccination of the recombinant adenoviral vector as described induces specific 3C cellular immune responses and thus provides broad cross-protection against different species of enteroviruses, including at least enterovirus 71 and coxsackievirus A.

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Patent status: US10004796, CN106794239A (Pending), KR20170068410A (Pending), SG11201609062PA (Pending), TW201602347A (Pending)

## Sorbitan polyester conjugates for stabilizing water-in-oil emulsions and delivering controlled release of bioactive agents

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Two key challenges in vaccine development are related to developing formulations that do not require refrigeration and finding an absorbable adjuvant in vivo. The former maintains and preserves the quality of a vaccine before it is administered, and the latter is critical to evading hypersensitive reactions after vaccine administration.

This invention discloses a W/O/W emulsion comprising SPAN® 85 andpoly(ethylene gjycoI)-bloi -poly(lactide-co-€-caprolactone) (PEG~b~PLACL) as an emulsifying agent. That emulsion system exhibited a controlled release of antigen similar to SPAN/TWEEN emulsion. However for a W/O/W emulsion, having the same controlled release effect does not translate into having the same emulsion-stabilizing effect. Moreover, it has been well documented that the traditional emulsifiers TWEEN®, SPAN® and poloxamers may cause toxicities including severe non-immunological anaphylactoid reactions.

The present invention discloses a composition in a water-in-oil-in-water (W/O/W) emulsion. The composition comprises: (a) a continuous aqueous phase, comprising H2O; (b) an oil phase or an oil shell, dispersed in the continuous aqueous phase; and (c) a hydrophilic polymer, stabilizing an interface between the continuous aqueous phase and the oil phase or the oil shell to form the water-in-oil-in-water (W/O/W) emulsion. The oil phase or the oil shell comprises: (i) oil; (ii) an internal aqueous phase, dispersed within the oil or the oil shell; and (iii) a lipophilic sorbitan-polyester conjugate, stabilizing an interface between the oil and the inner aqueous phase to form a water-in-oil (W/O) emulsion. The lipophilic sorbitan-polyester conjugate comprises: (1) sorbitan; and (2) poly(lactide-co-ε-caprolactone) or polylactic acid (polylactide), conjugated to the sorbitan.

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Patent status: TWI383806, TWI598114, US10172945, AU2016215757, CN107405304A (Pending)

## Lipidated survivin and the use thereof for prevention and treatment of cancers

Inventors: Hsin-Wei Chen, Chih-Hsiang Leng, Shih-Jen Liu, Pele Choi-Sing Chong

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The goal of tumor immunotherapy is aimed at activating the body's own immune defense system to fight and then to eliminate the existing tumor. However, immunotherapy has encountered a number of inherent difficulties in achieving a successful cancer treatment. They include the antigenic resemblance between the tumor and normal cells, the rapid growth of tumor cells with low immunogenicity, and the evasive nature of tumor cells to escape immune surveillance. The production of lipoproteins for enhancing the antigenicity of recombinant proteins has been a promising approach to develop novel vaccines.

Survivin is a member of inhibitor of apoptosis (IAP) family of proteins that prevents apoptosis and promotes cell proliferation. It has been shown that the expression of survivin is highly tumor specific. Survivin is over-expressed in several human cancers, including lung, breast, prostate, and ovarian cancers. Survivin is a growth factor-inducible gene that is strongly overexpressed in actively dividing endothelial cells forming blood vessels, and plays an important role in offsetting apoptotic stimuli and stabilizing the vascular network. Thus, it can be a universal target antigen for cancer immunotherapy

The present invention relates to isolated recombinant human lipidated survivin expressed in an *Escherichia coli*-based system. The isolated recombinant human lipidated survivin augments the expression levels of costimulatory molecules (MHC II, CD40, CD80, CD83, and CD86) and cytokines (IL-6, IL-12, and TNF-[alpha]). The results in the TC-1 tumor cell challenged animal model and the HLA-A11 transgenic mice suggest that the isolated recombinant human lipidated survivin can elicit immune responses against tumor growth and may be applied to survivin expressed tumor.

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Patent status: TWI628189