ID No.	K3012
Project Title	Development of M2e-based intranasal universal influenza vaccine utilizing PilVax platform
Principal Investigator	Catherine Tsai (Research Fellow, Univ. of Auckland)
Project Members IMSUT Host Researcher	Kohtaro Fujihashi (Project Prof., IMSUT)
Members	Hideki Asanuma (Chief, National Institute of Infectious Diseases)

Report

We have developed PilVax into a vaccine against influenza A virus infections. We showed that PilVax was able to present either one or two copies (in tandem) of the highly conserved epitope of influenza matrix 2 protein (M2e) efficiently on the surface of the recombinant *L. lactis* strains (Table 1). The recombinant *L. lactis* was generated in Auckland and shipped as frozen heat-killed aliquots from University of Auckland to IMSUT by using World Courier. The package (frozen) went through the custom on July 28, 2020 and delivered to Dr. Fujihashi's laboratory next day (please see attached document).

Enumerataion (colony counts)														
Strain	-4			Average	Actual (CFU/ml)	-5			Average	Actual (CFU/ml)	-6			Average	Actual (CFU/r
PilM1	39	28	36	34.33333	3.43E+07	12	11	13	12	1.20E+08	5	2	7	4.666667	4.67E+08
M2e	32	35	29	32	3.20E+07	13	17	13	14.33333	1.43E+08	6	6	8	6.666667	6.67E+08
M2e-M2e	41	39	50	43.33333	4.33E+07	10	14	12	12	1.20E+08	6	5	6	5.666667	5.67E+08

Due to pandemic COVID-19, Dr. Tsai could not visit the IMSUT and thus, Drs. Asanuma and Fujihashi have decided to initiate proposed experiments using vaccine antigens obtained from Dr. Tsai.

Four groups of mice were nasally immunized with either M2e-PilVax, M2e-M2e (tandem) PilVax (1 x 10^5) in the presence or absence of cholera toxin (CT, 1 μg) as nasal adjuvant or 1μg of inactivated whole influenza vaccine three times at weekly intervals. Eleven days after the last nasal immunization, all groups of mice were nasally challenged with influenza virus (Cal7: A/H1N1pdm09, $5LD_{50}$,50μl). Both groups of mice given M2e-PhiVax with CT and tandem PhilVax with CT showed significant protection against lethal challenge with Cal7 (Fig.1). However, both PhilVax without CT as nasal adjuvant failed to provide the protection. These results show that low doses of PhilVax require nasal adjuvant to elicit protective immunity.

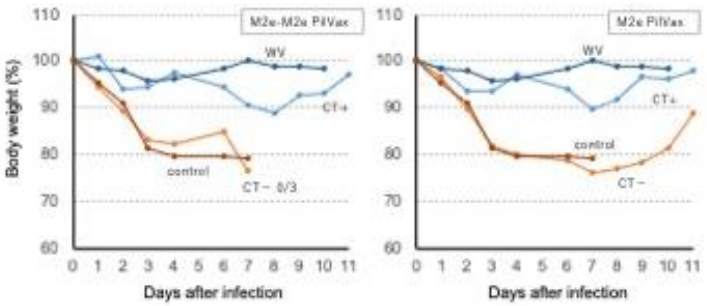

Figure 1

Dr. Tsai is scheduled to give a seminar outlining the project on December11, 2020 via the Zoom.

Thus far, we had two Zoom meetings and 49 (with Dr. Tsai) and 29 (with Dr. Asanuma) e-mail discussions. Further, Dr. Asanuma visited IMSUT and performed preparation of experiments and performed preliminary experiments.