RGC Ref.: A-CUHK401/14
(please insert ref. above)

The Research Grants Council of Hong Kong
ANR/RGC Joint Research Scheme
Completion Report

(Please attach a copy of the completion report submitted to the ANR by the French researcher)

Part A: The Project and Investigator(s)

1. Project Title (ANR Acronym)

Refining the bioactivity of P42, a hit therapeutic peptide, and developing a combined therapeutic peptide approach for treating Huntington's Disease. (PEP-FOR-HD)

2. Investigator(s) and Academic Department/Units Involved

	Hong Kong Team	French Team
Name of Principal Investigator *(with title)*	Dr. CHAN, Ho Yin Edwin	Dr. Florence Maschat
Post	Professor	Research Director
Unit / Department / Institution	School of Life Sciences, The Chinese University of Hong Kong	U710 Unit/ University of Montpellier 2
Contact Information	hyechan@cuhk.edu.hk	florence.maschat@umontpellier.fr
Co-investigator(s) *(with title and institution)*	Dr. CHAU, Ying Dr. KWAN, King Ming Dr. NGO, Jacky Dr. ZUO, Joan	Dr. LARROQUE, Christian Dr. MAUREL, Patrick Prof. VIGNES, Michel

3. Project Duration

	Original	Revised	Date of RGC/ Institution Approval *(must be quoted*)
Project Start date	01/04/2015		
Project Completion date	31/03/2019		
Duration *(in month)*	48		
Deadline for Submission of Completion Report	30/09/2020		

Part B: The Completion Report

5. Project Objectives

5.1 Objectives as per original application

1. Biophysical characterization and peptide engineering of P42;

2. *In vitro* and *in vivo* characterization of the suppression mechanisms of P42;
3. Investigation of the suppression effect of simultaneously delivering P42 and an RNA toxicity inhibitory peptide, P3, on HD disease models;
4. Development of non-invasive drug delivery systems for P42 and P3 peptides to the brain

5.2 Revised Objectives

Date of approval from the RGC: N/A

Reasons for the change: _____

_____

6. Research Outcome

Major findings and research outcome
(maximum 1 page; please make reference to Part C where necessary)

Major findings of the HK team are summarized below:

1) We previously showed that a peptidyl inhibitor, P3, which binds directly to expanded CAG RNA can inhibit RNA-induced nucleolar stress and suppress RNA-induced neurotoxicity. We performed peptide engineering on P3, and found that a N-acetylated and C-amidated derivative of P3, P3V8, that showed a more than 20-fold increase in its affinity for expanded CAG RNA. The P3V8 peptide also more potently alleviated expanded RNA-induced cytotoxicity in vitro, and suppressed polyQ neurodegeneration in Drosophila with no observed toxic effects. Further N-palmitoylation of P3V8 (L1P3V8) not only significantly improved its cellular uptake and stability, but also facilitated its systemic exposure and brain uptake in rats via intranasal administration. Our findings demonstrate that concomitant N-acetylation, C-amidation and palmitoylation of P3 significantly improve both its bioactivity and pharmacological profile. L1P3V8 possesses drug/lead-like properties that can be further developed into a lead inhibitor for the treatment of polyQ diseases (SEQ#3).

2) We further developed a brain-targeting delivery system for both QBP1 (protein toxicity peptide inhibitor) and L1P3V8 and evaluated their therapeutic effects on the R6/2 transgenic mouse model of Huntington's Disease (HD). Compared with intravenous administration, intranasal administration of QBP1 significantly increased its brain-to-plasma ratio. In addition, employment of a chitosan-containing in situ gel for the intranasal administration of QBP1 notably improved its brain concentration for up to 10-fold. Further study on intranasal cotreatment with the optimized formulation of QBP1 and L1P3V8 in mice found no interference on the brain uptake of each other. Subsequent efficacy evaluation of QBP1 and L1P3V8 intranasal co-treatment in the R6/2 mice demonstrated a significant improvement on the motor coordination and explorative behavior of the disease mice, together with a full suppression on the RNA- and protein-toxicity markers in their brains. This demonstrates that our intranasal co-treatment strategy is effective on treating mouse model of HD (SEQ#5).

3) During the course of our investigation, we further identified a novel CAG RNA toxicity peptide inhibitor a.k.a. BIND (see above). The bioactivity of BIND is 600% more effective than P3. We further demonstrated that BIND is capable of neutralizing other repeat RNA toxicity (SEQ#6).

Potential for further development of the research and the proposed course of action *(maximum half a page)*

Both the French team and Hong Kong collaborated on P42/P3 combined treatment studies. Unpublished results can be found on page 12 of the French team's final report (deliverables III and IV).

Besides, the Hong Kong team developed a nanoparticle (NP)-based delivery system to transfer peptide inhibitors to cells (1). Further adopting this emerging NP-mediated delivery system of peptides, e.g. BIND and P42, will be one potential for further development.

(1) Kim, M.R., Feng, T., Zhang, Q., **Chan, H.Y.E.** and Chau, Y. (2019) Co-encapsulation and co-delivery of peptide drugs via polymeric nanoparticles. Polymers **11**(2), 288

This is an extremely fruitful project both in terms of research publications, as well as the development of next generation peptide inhibitors and drug delivery system.

7. The Layman's Summary

(describe in layman's language the nature, significance and value of the research project, in no more than 200 words)

Huntington's Disease (HD) is a hereditary neurological disease caused by uncontrolled CAG triplet repeat expansion in the *IT15* locus of the human genome. Once expressed, the *HD* gene transcribes toxic RNA which subsequently produces disease protein in neurons. Both RNA and protein trigger neuronal cell death. The Hong Kong and French teams developed peptide inhibitors that target neurotoxic RNA and protein respectively. In this study, both teams worked independently but cooperatively to develop an effective strategy to combat HD. We (Hong Kong team) validated the co-peptide treatment in a HD mouse disease model. When compared to single peptide treatment, co-treating disease mice with both P3 and QBP1 peptide inhibitors resulted in a more effective therapeutic effect. Further, we developed a new generation of RNA toxicity candidate drug, a.k.a. BIND, which can neutralize toxic RNAs 600X more effective than the original P3. Together with the research outcomes obtained from the French team, we demonstrated that peptide therapeutics is an attractive alternative option for HD treatment.

Part C: Research Output

8. Peer-reviewed journal publication(s) arising directly from this research project

(Please attach a copy of each publication and/or the letter of acceptance if not yet submitted in the previous progress report(s). All listed publications must acknowledge RGC's funding support by quoting the specific grant reference.)

The Latest Status of Publications				Author(s) ***(bold** the authors belonging to the project teams and denote the corresponding author with an asterisk*)*	Title and Journal/ Book *(with the volume, pages and other necessary publishing details specified)*	Submitted to RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknowledged the support of this Joint Research Scheme *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
Year of publication	Year of Acceptance *(For paper accepted but not yet published)*	Under Review	Under Preparation *(optional)*						

2017 (#1)				Zhang,Q., Tsoi, H., Peng, S.H., Li, P.P., Lau, K.F., Rudnicki, D.D., **Ngo, J.C.** and **Chan, H.Y.E.***	Assessing a peptidylic inhibitor-based therapeutic approach that simultaneously suppresses polyglutamine RNA- and protein-mediated toxicities in patient cells and *Drosophila*. Dis. Model. Mech. **9**, 321-334.	2017	No	Yes	Yes
2017 (#2)				Koon, A.C. and **Chan H.Y.E.***	*Drosophila melanogaster* as a model organism to study RNA toxicity of repeat expansion-associated neurodegenerative and neuromuscular diseases. Front. Cell. Neurosci. **11**:70. *doi: 10.3389/fncel.2017.00070*	2017	No	Yes	Yes

2017 (#3)				Zhang, Q., Yang, M., Sorensen, K.K., Madsen, C.S., Boesen, J.T., An, Y., Peng, S.H., Wei, Y., Wang, Q., Jensen, K.J., Zuo, Z., **Chan, H.Y.E.*** and Ngo, J.C.K.*	A brain-targeting lipidated peptide for neutralizing RNA-mediated toxicity in polyglutamine diseases. Sci. Rep. **7**(1): 12077	2020	Yes	Yes	Yes
2018 (#4)				Zhang, Q., Chan, Z.S., An, Y., Liu, H., Hou, Y., Li, W., Lau, K.F., Koon, A.C., Ngo, J.C.K.* and **Chan, H.Y.E.***	A peptidylic inhibitor for neutralizing expanded CAG RNA-induced nucleolar stress in polyglutamine diseases. RNA **24**, 486-498.	2020	Yes	Yes	Yes
2018 (#5)				Yang, M., Zhang, Q., Wang, Q., Sorensen, K.K., Boesen, J.T., Ma, S.Y., Jensen, K.J., Kwan, K.M., Ngo, J.C.K., **Chan, H.Y.E.** and Zuo, Z.	Brain-targeting delivery of two peptidylic inhibitors for their combination therapy in transgenic polyglutamine disease mice via intranasal administration. Mol. Pharm. **15**, 5781-5792.	2020	Yes	Yes	Yes

2019 (#6)				Zhang, Q., An, Y., Chen, Z.S., Koon, A.C., Lau, K.F., Ngo, J.C.* and **Chan, H.Y.E.***	A peptidylic inhibitor for neutralizing r(GGGGCC)exp-associated neurodegeneration in C9ORF72-associated amyotrophic lateral sclerosis and frontotemporal dementia. Mol. Ther. Nucleic Acids **16**, 172-185.	2020	Yes	Yes	Yes

9. Recognized international conference(s) in which paper(s) related to this research project was/were delivered *(Please attach a copy of each delivered paper. All listed papers must acknowledge RGC's funding support by quoting the specific grant reference.)*

Month/Year/ Place	Title	Conference Name	Submitted to RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknowledged the support of this Joint Research Scheme *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
N/A						

10. Student(s) trained *(Please attach a copy of the title page of the thesis.)*

Name	Degree registered for	Date of registration	Date of thesis submission/ graduation
N/A			

11. Other impact *(e.g. award of patents or prizes, collaboration with other research institutions, technology transfer, etc.)*

- Chan, H.Y.E.; Ngo, J.C.; Zhang, Q. Peptidylic inhibitors targeting C9ORF72 hexanucleotide repeat-mediated neurodegeneration. U.S. Patent US-2018-0214515-A1, August 2, 2018

- Chan, H.Y.E.; Ngo, J.C.; Zhang, Q. Peptidylic inhibitor targeting CAG-repeat RNA toxicity in polyglutamine diseases. U.S. Patent US-2017-0233442-A1, August 17, 2017

- Chan, H.Y.E.; Jensen, K.J.; Ngo, J.C.; Sorensen, K.K.; Zhang, Q.; Zuo, Z. P3 Peptidylic inhibitors for treating CAG-repeat RNA toxicity in polyglutamine diseases. U.S. Patent 62/465,513, March 10, 2017

- Chan, H. Y. E.; Tsoi, H.; Ngo, J. C.; Lau, K. F. Therapeutic approach for polyglutamine degeneration. U.S. Patent US 9,297,798, March 29, 2016

12. Statistics on Research Outputs *(Please ensure the summary statistics below are consistent with the information presented in other parts of this report.)*

	Peer-reviewed journal publications	Conference papers	Scholarly books, monographs and chapters	Patents awarded	Other research outputs (Please specify)
No. of outputs arising directly from this research project	6	0	0	4	0