RGC Ref.: **A-HKUST601/13**
(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)

Title: Calcium signalling in glioblastoma multiforme: Combining oncology and neurogenesis with synthetic biology and optical imaging to relate calcium signalling with neural stemness.
ANR Acronym: CalciumGlioStem

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

	Hong Kong Team	French Team
Name of Principal Investigator *(with title)*	Prof. Andrew L. Miller	Dr Catherine Leclerc
Post	Professor	Chargée de Research 1st Class, CNRS
Unit / Department / Institution	Division of Life Science, HKUST	University of Toulouse CNRS UPS (Formerly known as CNRS/UMR 5547, Université Paul Sabatier)
Contact Information	Tel: 2358 8631 E-mail: almiller@ust.hk	Tel: 05 61 55 63 98 E-mail: catherine.leclerc@univ-tlse3.fr
Co-investigator(s) *(with title and institution)*		Dr Marc Moreau/ University of Toulouse CNRS UPS (Formerly known as Université Paul Sabatier). Dr Hervé Chneiweiss/ CNRS Neurosciences Paris-Seine, Paris. Prof. Jacques Haiech/ Université Louis Pasteur – Laboratoire d'Innovation Thérapeutique, Strasbourg. Prof. Marie-Claude Kilhofer/ Université Louis Pasteur – Laboratoire d'Innovation Thérapeutique, Strasbourg.

3. Project Duration

	Original	Revised	Date of RGC/ Institution Approval *(must be quoted*)
Project Start date	10/01/2014	N/A	
Project Completion date	31/12/2016	30/06/2017	06/09/2016 Institution approval
Duration *(in month)*	36	42	06/09/2016 Institution approval
Deadline for Submission of Completion Report	31/12/2017	30/06/2018	

Part B: The Completion Report

5. Project Objectives

5.1 Objectives as per original application:

1. To understand how Ca^{2+} controls the stem cell state properties of GBM and via which Ca^{2+} toolkit components.

2. To investigate how abnormalities in Ca^{2+} homeostasis affects the dynamics and progression of GBM-related properties.

5.2 Revised Objectives: **N/A**

Date of approval from the RGC: _____

Reasons for the change: _____

1.
2.
3.

6. Research Outcome

Major findings and research outcome (*maximum 1 page*)

The Miller lab has been involved mainly in the zebrafish-based experiments in Task 2 of the project. Thus, we: 1) Successfully developed lines of transgenic fish, which stably express the bioluminescent Ca^{2+} indicators aequorin or nano-lantern under the control of the *her4.1* promoter; 2) Investigated the role of Ca^{2+} signaling during normal neurogenesis in neural stem cells (NSCs) isolated from the brain of adult zebrafish; and 3) Developed an electroporation method to introduce apoaequorin into zebrafish NSCs in suspension culture.

1) Generating transgenic zebrafish fish lines where a variety of bioluminescent Ca^{2+} reporters have been expressed under the control of the *her4.1* promoter. A significant period was spent overcoming technical challenges that are the norm with regards to innovative and complex research projects. The generation of viable transgenic vertebrates (including zebrafish) that do not suffer from abnormalities resulting from either the techniques used or the foreign genes introduced is a challenging and time-consuming process, which takes many months to achieve a stable line. However, where transgenesis is successful, the end-results are well worth the effort. In this case, two lines of F_2 homozygous transgenic fish which stably express the bioluminescent Ca^{2+} indicators GFP-aequorin or nano-lantern Ca^{2+} 150 under the control of the *her4.1* promoter have been successfully generated and stable lines of both have been developed.

2) Identification of Ca^{2+} signaling components in proliferating and differentiating neurospheres derived from primary neural stem cells isolated from the zebrafish brain. We used a variety of complementary methods to investigate the role of Ca^{2+} signaling during normal neurogenesis in neural stem cells isolated from the brains of wild-type adult fish. The methods used and results obtained are outlined below:

(a) Isolating primary NSCs from adult zebrafish brains via neurosphere production. The development of reliable protocols for isolating/culturing neural adult stem/progenitor cells from the brain of adult fish (Cortés-Campos *et al*., 2015), meant that a detailed analysis of the molecular mechanisms underlying neurogenesis was possible. Using similar protocols, we demonstrated that cells dissociated from neurospheres can differentiate into cells with neuronal, astrocyte or oligodendrocyte characteristics. We subsequently used neurospheres (and cells dissociated from neurospheres) at proliferation day 5 (P5) and differentiation days 7 and 14 (D7 and D14) to identify the Ca^{2+} signaling dynamics and Ca^{2+} toolkit components responsible for the maintenance and switch between proliferation and differentiation in NSCs. **(b) The mRNA levels of selected Ca^{2+} toolkit elements in NSCs and NSC-derived cells are modulated during the switch from proliferation to differentiation states.** To identify Ca^{2+} toolkit components that are expressed during proliferation and differentiation, mRNA was extracted from NSCs and NSC-derived cells at P5, D7 and D14. Subsequent qPCR showed that at D14, there was a significantly reduced level of *ryr2a* mRNA but significantly higher levels of *ip3r1b, ip3r3, ryr3*, and *stim1b* mRNA. **(c) The**

Ca^{2+} chelator, BAPTA-AM, significantly reduces the elongation of outgrowths in differentiating cells. P5 cells were plated and cultured in differentiation medium in the presence or absence of the membrane permeable intracellular Ca^{2+} chelator, BAPTA-AM, for 7 days and the morphology of the cells was then analyzed. Our data showed that the total length of outgrowths in the differentiating cells treated with BAPTA-AM was significantly shorter than those in the solvent controls, whereas there was no significant difference in the number of outgrowths per cell.
(d) Expression and localization of IP_3R1 & RyR2 in cells with neuronal and glial morphology at D7. Immunolabelling experiments were conducted to investigate the localization of IP_3R1 and RyR2 in cells expressing HuC/D (a neuronal marker) and GFAP (a glial marker) at D7. Our results indicated that IP_3R (type 1) and RyR (type 2) are expressed in cells with neuronal- or glial-like properties but with different patterns of localization. **(e) Effect of ATP and caffeine on the stimulation of Ca^{2+} signals generated by dissociated 2° neurosphere cells with neuron- or glial-like morphology.** We showed that dissociated P5 and D7 cells that exhibited neuron- or glia-like morphologies generated intracellular Ca^{2+} signals in response to stimulation by ATP but not caffeine. ***A manuscript describing the results obtained for #2, is currently under review in Science China Life Science – see Tse et al. in Section C8.***
3) Development of an electroporation method to introduce apoaequorin into zebrafish neural stem cells. Dr. Francisco Aulestia from the lab of the French PI, Dr. Leclerc, spent 3 weeks during Fall 2016, working in Prof Miller's lab at HKUST where he helped to develop methods to optimize the incorporation (via electroporation) of EGFP-apoaequorin into primary cultures of cells dissociated from the zebrafish brain. This is a technique that Dr. Aulestia used routinely for his work on human GBM cells in the Toulouse-based laboratory. ***A manuscript describing the work conducted for Research Outcome #3, is currently in preparation – see Aulestia et al. in Section C8.***

Reference:
Cortes-Campos, C., Letelier, J., Ceriani, R., Whitlock, K.E. (2015). Zebrafish adult-derived hypothalamic neurospheres generate gonadotrophin-releasing hormone (GnRH) neurons. Biol Open 4, 1077-1086.

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

The lines of homozygous transgenic fish that have been generated, which stably express the bioluminescent Ca^{2+} indicators GFP-aequorin or nano-lantern Ca^{2+} 150 under the control of the *her4.1* promoter, are now ready for further use in experiments to investigate the pattern of Ca^{2+} signaling in the normally developing/functioning brain, as well as how the Ca^{2+} signals might change with GBM. We plan to make these fish lines available on-request to other researchers. We have the pDEST-gfap:Gal4-vp16 and pIUI-mCherry-$KRas^{G12V}$ constructs from Dr Michael Taylor (St Jude Children's Research Hospital, Memphis, TN, USA), which when injected into early zebrafish embryos induce brain tumors. It is reported that microinjection of these constructs leads to around 20-50% of the fish carrying brain tumors, some of which are GBM (Ju *et al*., 2015). Brain tumors can be identified by fluorescence immunolabeling cells with the CD133 primary antibody. Brain tumor cells can also be purified by fluorescence-activated cell sorting (FACS) and neurospheres subsequently prepared. The Ca^{2+} signals generated in the brain of live transgenic fish that stably express aequorin or nano-lantern Ca^{2+} and transiently express $KRas^{G12V}$ or in neurospheres isolated from the brains of aequorin/nano-lantern and $KRas^{G12V}$-expressing fish can be studied. The presence of tumor cells and specifically the manifestation of GBM can be identified retrospectively in intact fish and in isolated cells as appropriate. This data obtained can be compared with data generated from normal (i.e., without transient KRas expression) transgenic fish that stably express aequorin or nano-lantern Ca^{2+} as controls. Once the Ca^{2+} signals in normal and GBM brains or neurospheres are characterized, the effect of treatment of GBM cells with bisacodyl can be determined.

Reference:
Ju, B., Chan, W., Orr, B.A., Spitsbergen, J.M., Jia, S., Eden, C.J., Henson. H.E., and Taylor, M.R. (2015). Oncogenic KRAS promotes malignant brain tumors in zebrafish. Mol. Cancer 14:18 DOI: 10.1186/s12943-015-0288-2.

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)

The development of the central nervous system (CNS) in vertebrate embryos involves the generation of different types of neurons and glia in a complex but highly-ordered spatio-temporal manner. Zebrafish are commonly used for exploring the formation/plasticity/regeneration of the CNS, and the recent development of reliable protocols for isolating and culturing neural stem/progenitor cells from the brain of adult fish now enables the exploration of mechanisms underlying the induction/specification/differentiation of these cells. In this project, we refined a protocol to generate proliferating and differentiating neurospheres from the brain of adult zebrafish. We demonstrated that: the genes that encode some Ca^{2+} channels (*ip3r*, *ryr* and *stim)* are significantly upregulated or downregulated in differentiating neurospheres; the endoplasmic reticulum-based Ca^{2+} channels, 1,4,5-inositol trisphosphate receptor type-1 and ryanodine receptor type-2 are differentially expressed in cells with neuron- or radial glial-like properties; and ATP (an IP_3R agonist) induced the generation of Ca^{2+} transients in these cells whereas caffeine (an RyR agonist), did not. Our results indicate the differential expression of Ca^{2+}-signaling components in proliferating and differentiating cells. Thus, given the complexity of the intact vertebrate brain, neurospheres might be useful for exploring glioblastoma multiforme diagnosis protocols and drug development using Ca^{2+}-signaling as a read-out.

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.)

MULTI-PARTNER PUBLICATIONS

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)*						
	2018			M.K. Tse, T.S. Hung, C. M. Chan, T. Wong, M. Dorothea, **C. Leclerc, M. Moreau, A.L. Miller** and **S.E Webb***	Identification of Ca^{2+} signaling components in neural stem/progenitor cells during differentiation into neurons and glia in intact and dissociated zebrafish neurospheres. *Sci China Life Sci Doi: 10:1007/s11427-018-9315-6.*	2018	Yes	Yes	No

	2018			H.B. Hao, **S.E. Webb,** J. Yue, **M. Moreau, C. Leclerc** and **A.L. Miller***	The effect of pyrazole 3 treatment on the generation of intracellular Ca^{2+} transients and mitochondrial membrane potential, as well as the survival and neural differentiation of mouse embryonic stem cells. *J. Stem Cells (In press)*	2018	Yes	Yes	No
2018				H.B. Hao, **S.E. Webb,** J. Yue, **M. Moreau, C. Leclerc, and A.L. Miller***	TRPC3 is required for the survival, pluripotency and neural differentiation of mouse embryonic stem cells (mESCs). *Sci. China Life Sci. 61(3):253-265.*	2018	Yes	Yes	Yes
2016				**C. Leclerc, J. Haiech,** F. Aulestia, **M.-C. Kilhoffer, A.L. Miller, I. Néant, S.E. Webb**, E. Schaeffer, M.P. Junier, **H. Chneiweiss**, and **M. Moreau***	Calcium signaling orchestrates glioblastoma development: Facts and conjunctures. *Biochim. Biophys. Acta 1863: 1447-1459.*	2018	Yes	Yes	Yes

2016				**Moreau, M., Néant, I., Webb, S.E**., **Miller, A.L**., Riou, J.-F., **Leclerc, C**.*	Ca^{2+} coding and decoding strategies for the specification or neural and renal precursor cells during development. *Cell Calcium*. 59(2-3): 75-83.	2018	Yes	Yes	Yes
			2018	**F.J. Aulestia,** C.M. Chan, M.K. Tse, **C. Leclerc**, **M. Moreau, S.E. Webb, A.L. Miller***	Monitoring Ca^{2+} signals in the cytosol and mitochondria of both single cells and multi-cellular neurospheres derived from the adult zebrafish brain. (*In revision).*	2018	Yes	Yes	No
			2018	**I. Néant,** H.C. Leung, **S.E. Webb, A.L. Miller, M. Moreau C. Leclerc***	TRPC1 is the missing link between the BMP and the Ca^{2+} signaling pathways during neural specification in amphibians. (*Working paper).*	2018	Yes	Yes	No

	2017			**S.E. Webb, A.L. Miller***	Ca^{2+} signaling and membrane dynamics during cytokinesis in animal cells. In Press in *Advances in Experimental Medicine and Biology*, Vol. 981, Joachim Krebs (Eds): Membrane Dynamics and Calcium Signaling.	2018	Yes	Yes	No
	2018			**S.E. Webb*, A.L. Miller**	The use of complementary luminescent and fluorescent techniques for imaging Ca^{2+} signalling events during the early development of zebrafish (*Danio rerio*) *Invited Book* Chapter In Press in *Methods in Molecular Biology*. Claus Heizmann (Ed.)	2018	Yes	Yes	No

2018				J.J. Kelu, **S.E. Webb,** A. Galione, **A.L. Miller***	TPC2-mediated Ca^{2+} signaling is required for the establishment of synchronized activity in developing zebrafish primary motor neurons. *Dev. Biol*. 438: 57-68.	2018	Yes	Yes	Yes
2017				J.J. Kelu, **S.E. Webb,** J. Parrington, A. Galione, **A.L. Miller***	Ca^{2+} release via two-pore channel type 2 (TPC2) is required for slow muscle cell myofibrillogenesis and myotomal patterning in intact zebrafish embryos. *Dev. Biol.* 425:109-129.	2018	Yes	Yes	Yes

2016				C.M. Chan, J.T.M. Aw, **S.E. Webb**, **A.L. Miller***	SOCE proteins, STIM1 and Orai1, are localized to the cleavage furrow during cytokinesis of the first and second cell division cycles in zebrafish embryos. *Zygote 24:880-889*.	2018	Yes	Yes	Yes
2016				H.Y.S. Chan, M.C. Cheung, Y. Gao, **A.L. Miller, S.E. Webb***	Expression and reconstitution of the biolumines cent Ca^{2+} reporter aequorin in human embryonic stem cells, and exploration of the presence of functional IP_3 and ryanodine receptors during the early stages of their differentiation into cardiomyocytes. *Sci. CHINA Life Sci. 59(8): 811-824.*	2018	Yes	Yes	Yes

2015				J.J. Kelu, H.L.H. Chan, **S.E. Webb,** A.H.H. Cheng, M. Ruas, J. Parrington, A. Galione, **A.L. Miller***	Two-pore channel 2 activity is required for slow muscle cell-generated Ca^{2+} signaling during myogenesis in intact zebrafish. *Int. J. Dev. Biol*. 59: 313-325.	2018	Yes	Yes	Yes
2015				M. Barnes, G. van Rensburg, W.M. Li, K. Mehmood, S. Mackedenski, C.M. Chan, D.T. King, **A.L. Miller**, C.H. Lee*	Molecular insights into the coding region determinant-binding protein-RNA interaction through site-directed mutagenesis in the heterogeneous nuclear ribonucleoprotein-K-homology domains. *J. Biol. Chem. 290:625-639.*	2015	No	Yes	Yes
2015				C.M. Chan, Y. Chen, T.S. Hung, **A.L. Miller**, A.M. Shipley, **S.E. Webb***	Inhibition of SOCE disrupts cytokinesis in zebrafish embryos via inhibition of cleavage furrow deepening. *Int. J. Dev. Biol*. 59: 289-301.	2018	Yes	Yes	Yes

			2018	C. Dedic, T.S. Hung, A.M. Shipley, A. Maeda, T. Gardella, **A.L Miller**, P. Divieti Pajevic, J.G. Kunkel, A. Rubinacci*	Calcium fluxes at the bone/plasma interface: acute effects of parathyroid hormone (PTH) and targeted deletion of PTH/PTH-related peptide (PTHrP) receptor in the osteocytes. *Bone (under review)*	2018	Yes	Yes	No
			2018	L. Yang, **S.E. Webb**, N. Jin, H.M. Lee, T.F. Chan, G. Xu, J.C.N. Chan, **A.L. Miller***, R.C.W. Ma*	Characterization of the key role of *dachshund b* in the development of the pancreatic islet in zebrafish (*Danio rerio*) *Manuscript in preparation*	2018	Yes	Yes	No

		2018		**S.E. Webb***, J.J. Kelu, **A.L. Miller**	Investigating the role of two pore channel 2 (TPC2) in zebrafish neuromuscular development. *Invited review in CRC Methods in Signaling Transduction Series entitled "Ion and Molecule Transport in Lysosomes (Under Review).*	2018	Yes	Yes	No
			2018	J.T. Hung, **S.E. Webb,** C. Palumbo, A. Lesniak, A.M. Shipley, A. Rubinacci, J.G. Kunkel, **A.L. Miller***	Investigating the role played by the elasmoid scales of zebrafish (*Danio rerio*) in the short-term homeostatic regulation of extracellular fluid/plasma Ca^{2+} levels. (*Working paper*)	2018	Yes	Yes	No

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)*
October 2017 / Awaji, Japan	Identification of Ca^{2+} signaling components in proliferating and differentiating neurospheres derived from primary neural stem cells isolated from the zebrafish brain.	20th Ca^{2+} Binding Proteins and Ca^{2+} Function in Health and Disease conference (CaBP20)	2018	Yes	Yes	No
October 2017 / Awaji, Japan	TPC2-mediated Ca^{2+} signaling is required for the establishment of synchronized connectivity within the zebrafish embryonic spinal circuitry	20th Ca^{2+} Binding Proteins and Ca^{2+} Function in Health and Disease conference (CaBP20)	2018	Yes	Yes	No
October 2017 / Awaji, Japan	TPC2-mediated Ca^{2+} signaling is required for the development of slow muscle cells in zebrafish embryos.	20th Ca^{2+} Binding Proteins and Ca^{2+} Function in Health and Disease conference (CaBP20)	2018	Yes	Yes	No
October 2017 / Awaji, Japan	Investigation of the role of Ca^{2+} signaling during early embryonic heart development in zebrafish.	20th Ca^{2+} Binding Proteins and Ca^{2+} Function in Health and Disease conference (CaBP20)	2018	Yes	Yes	No
October 2016/ Singapore	Investigating the use of zebrafish as a model to study glioblastoma multiforme: Identification of the Ca^{2+} signaling toolkit components that regulate the self-renewal and differentiation of neural stem cells isolated from the brain of adult fish.	The 9th Zebrafish Disease Models Conference	2018	Yes	Yes	No

July 2016/ Zunyi, China	TRPC3 appears to play a dual role in inducing mouse embryonic stem cells to differentiate along a neuronal lineage as well as supporting cell survival	The 11th Symposium on Calcium Signaling in China (SCSC)	2018	Yes	Yes	No
June 2016 / San Francisco, USA	An investigation of the role of TRPC3 in the neural differentiation of mouse embryonic stem cells (ESCs).	International Society for Stem Cell Research (ISSCR)	2018	Yes	Yes	No
May 2016 / Macau	Store-operated Ca^{2+} entry (SOCE) via TRPC3 is required for cell proliferation, differentiation, and survival of undifferentiated Sox1-GFP 46C mouse embryonic stem cells (mESCs) as well as those undergoing neural differentiation.	3rd Macau Symposium on Biomedical Sciences 2016	2018	Yes	Yes	No
May 2016 / Macau	An exploration of the molecular components of the Ca^{2+} signaling toolkit that regulate the self-renewal and differentiation of neural stem cells isolated from the brain of adult zebrafish.	3rd Macau Symposium on Biomedical Sciences 2016	2018	Yes	Yes	No
May 2016 / Macau	Morphometric analysis of human embryonic stem cell-derived ventricular cardiomyocytes: Determining the maturation state of a population by quantifying parameters in individual cells.	3rd Macau Symposium on Biomedical Sciences 2016	2018	Yes	Yes	No
May 2016 / Macau	Expression and reconstitution of the bioluminescent Ca^{2+} reporter, aequorin, in human embryonic stem cells.	3rd Macau Symposium on Biomedical Sciences 2016	2018	Yes	Yes	No

October 2015/ Hong Kong	The role of Ca^{2+} signaling in the onset and progression of glioblastoma multiforme.	Hong Kong - San Diego Workshop on Signaling	2018	Yes	Yes	No
October 2015/ Hong Kong	Investigating the orchestration of FGF/BMP/Ca^{2+} signaling in regulating neurogenesis of mouse embryonic stem cells (mESCs).	Hong Kong - San Diego Workshop on Signaling	2018	Yes	Yes	No
June/2015/ Oslo	Two-pore channel 2 activity is required for slow muscle cell generated Ca^{2+} signaling during myogenesis in intact zebrafish.	9th European Zebrafish Meeting	2015	No	Yes	No
June/2015/ Maine	TRPC1 and DHPR-sensitive Ca^{2+} channels play a role in early neurogenesis in *Xenopus laevis* embryos.	Gordon Research Conferences on Calcium Signalling: Molecular and Cellular Mechanisms in Health and Disease	2015	No	Yes	No
June/2015/ Maine	Investigating the orchestration of FGF/BMP/Ca2+ signaling in regulating neurogenesis of mouse embryonic stem cells (mESCs).	Gordon Research Conferences on Calcium Signalling: Molecular and Cellular Mechanisms in Health and Disease	2015	No	Yes	No
May/2015/ Hong Kong	Role of two-pore channel 2 activity in generating slow muscle cell Ca^{2+} signals during myogenesis in intact zebrafish.	Hong Kong Inter-University Postgraduate Symposium on Life Science 2015	2015	No	Yes	No
May/2015/ Hong Kong	The zebrafish scale: A potential model for studying Ca^{2+} homeostasis in vertebrates.	Hong Kong Inter-University Postgraduate Symposium on Life Science 2015	2015	No	Yes	No

May/2015/ Hong Kong	Investigating the role of XTRPC1 and XBMPRII in regulating Ca^{2+} signaling events during neural induction in *Xenopus laevis.*	Hong Kong Inter-University Postgraduate Symposium on Life Science 2015	2015	No	Yes	No
Jan/2015/ Hong Kong	The orchestration of FGF/BMP/Ca^{2+} signaling in regulating neurogenesis of mouse embryonic stem cells (mESCs).	The Croucher Foundation ASI on Stem Cells: Biology and Applications	2015	No	Yes	No
Nov/2014/ Suzhou	The zebrafish scale as a possible model for studying mammalian bone/plasma Ca^{2+} exchange.	Cold Spring Harbor Asia Conference: Bone and Cartilage: From Development to Human Diseases	2015	No	Yes	No
Nov/2014/ Suzhou	The zebrafish scale: a model for studying the molecular regulation of mammalian bone/plasma Ca^{2+} exchange: the immediate bone wounding response; and bone regeneration and repair.	Cold Spring Harbor Asia Conference: Bone and Cartilage: From Development to Human Diseases	2015	No	Yes	No
Sept/2014/ Aix-en-Provence	Knock-down and rescue of TPC2-mediated Ca^{2+} signaling required for myofibrillogenesis in zebrafish slow muscle cells.	13th International Meeting of the European Calcium Society	2015	No	Yes	No
Sept/2014/ Aix-en-Provence	An exploration of Ca^{2+} signaling during heart development in zebrafish	13th International Meeting of the European Calcium Society	2015	No	Yes	No

Sept/2014/ Aix-en-Provence	Early neurogenesis in the amphibian requires the activation of TRPC1 and DHP-Ca^{2+} channels	13th International Meeting of the European Calcium Society	2015	No	Yes	No
June/2014/ Hong Kong	The zebrafish scale as a possible model for studying bone / plasma Ca^{2+} exchange.	Hong Kong InterUniversity Biochemistry Postgraduate Symposium 2014	2015	No	Yes	No

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
LEUNG Ho Chi	MPhil	1st Feb, 2014	31st Jan, 2016
CHAN Yin Seng Harvey	MPhil	1st Feb, 2013	2nd Feb 2015
HUNG Tin Shing	MPhil	2nd Sept 2013	28th Jul 2016
KELU Jeffrey Jenkin	PhD	1st Sept 2014	28th Aug 2017

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

During the course of this project, we maintained ongoing collaborations with:
Prof Antony Galione (Dept. Pharmacology, Oxford University, UK)
Dr John Parrington (Dept. Pharmacology, Oxford University, UK)
Prof. David Whitmore (Dept. Cell and Developmental Biology, University College, London, UK)
Profs Bernard and Christine Thisse (Dept. Cell Biology, University of Virginia, VA, USA)
Dr Robert Baker (Dept. Physiology & Biophysics, NYUMC, New York, USA)
Drs Marc Moreau and Catherine Leclerc (CNRS, University Paul Sabatier, Toulouse, France)

In addition, we initiated new collaborations with:
Dr Jacques Haiech (University Louis Pasteur, Strasbourg, France)
Dr Hervé Chneiweiss (University Paris Descartes, Paris, France)
Dr Marie-Claude Kilhoffer (University Strasbourg, France)
Dr Jianbo Yue (Department of Medical Sciences, CityU, Hong Kong)
Prof. Juliana Chan and Dr. Ronald Ma (Hong Kong Institute of Diabetes and Obesity, CUHK, Hong Kong)
Dr Chevonne Angus (NAFC Marine Centre, University of Highlands and Islands, Scotland, UK)
Prof. Kenneth Boheler and Dr Ellen Poon (SCRMC, HKU, Hong Kong)
Dr Marko Horb (National *Xenopus* Resource, Marine Biological Laboratory, Woods Hole, MA, USA)
Prof. Claudio Alonso (School of Life Sciences, University of Sussex, UK)