RESEARCH GRANTS COUNCIL
THEME-BASED RESEARCH SCHEME (TRS)

Completion Report on Funded Project

Project start date: *1 December 2011*
Project completion date: *30 November 2016*

1. Project Title: Massively Parallel Sequencing of Plasma Nucleic Acids for the Molecular Diagnostics of Cancers

2. Names and Academic Affiliations of Project Team Members[#]

Project Team	Name / Post	Unit / Department / Institution	Average number of hours per week spent on this project in the current reporting period
Project Coordinator	Y.M. Dennis Lo / Associate Director Director Professor	State Key Laboratory in Oncology in South China Li Ka Shing Institute of Health Sciences Department of Chemical Pathology, The Chinese University of Hong Kong (CUHK)	5
(Co)-Principal Investigator(s)	Rossa W.K. Chiu / Professor	Department of Chemical Pathology & Li Ka Shing Institute of Health Sciences, CUHK	5

	Tony S.K. Mok / Professor	Department of Clinical Oncology, CUHK	2
	K.C. Allen Chan / Professor	Department of Chemical Pathology & Li Ka Shing Institute of Health Sciences, CUHK	8
#	Si Lok / Professor of Practice	Li Ka Shing Institute of Health Sciences, CUHK	
	Hao Sun / Associate Professor	Department of Chemical Pathology & Li Ka Shing Institute of Health Sciences, CUHK	3
	Winnie Yeo / Professor	Department of Clinical Oncology, CUHK	2
	Henry L.Y. Chan / Professor	Department of Medicine & Therapeutics, CUHK	2
Co-investigator(s)	Anthony T.C. Chan / Director Associate Director Professor	Sir Y.K. Pao Cancer Centre State Key Laboratory in Oncology in South China Department of Clinical Oncology, CUHK	
Collaborators	Paul B.S. Lai / Professor	Department of Surgery, CUHK	

 # Please highlight the approved changes in the project team composition and quote the date when the RGC granted approval of such changes. For changes in the project team composition, please submit a separate request, together with the justification and the curriculum vitae of the new member(s), to the RGC three months prior to the intended effective date of the change.

Professor Si Lok's contract with The Chinese University of Hong Kong ended in February 2014. There was no replacement for the post. The project team has since successfully set up the droplet digital polymerase chain reaction (PCR) system based on the Bio-Rad QX100 model. Thus, Professor Lok's departure has not led to any delay in the project goals. This had been reported in the progress report on stage 3 of the project.

3. Project Objectives

Summary of objectives addressed/achieved:

Objectives*	**Percentage Achieved**	**Remarks****
1. Generic non-invasive genome-wide scanning of cancer-associated molecular alterations in plasma.	1. 100%	1. Completed
2. Specific approaches for the detection of molecular signatures of HCC and NSCLC	2. 100%	2. Completed
3. Novel diagnostic parameters from massively parallel plasma nucleic acid sequencing analysis	3. 100%	3. Completed

** Please highlight the approved changes in objectives and quote the date when the RGC granted approval of such changes.*

******* *Please provide reasons for significantly slower rate of progress than originally planned.*

6. Research Highlights and Outputs

(Maximum 20 A4 pages for sections 6 to 9, excluding any appendices and attachments)

6.1 What are the most exciting research accomplishments of the project?

(Please list five or more of the team's best research accomplishments, such as journal and conference papers, software codes, research infrastructure, etc. For each item, please clearly justify how it has achieved international excellence (e.g. best paper award, invited presentation, citations, product licensed to industry, etc.))

1. **The first ever demonstration that analysis of circulating tumour-derived DNA (ctDNA) is effective for the screening of cancer among asymptomatic individuals recruited from the community.** Through the analysis of plasma EBV DNA, we achieved the detection of 34 nasopharyngeal cancers (NPCs) among 20,174 healthy participants. Based on historical data, the peak incidence was 8 (NPCs) among 20,000 individuals. 71% of the NPCs detected by screening were of early stage while only 20% of NPCs from a historical unscreened cohort would be of early stage. Upon yearly phone follow-up for a median of 22 months, we have identified one false-negative NPC presenting within one year of ctDNA screening. The screened cohort has significantly better 3-year progression free survival with hazard ratio of 0.10 (95% confidence interval, 0.05 to 0.18). The data show that sensitivity for NPC screening was 97.1% while the specificity was 98.6%. The positive predictive value was 11.0%. These results have been published in Aug 2017 in the top-ranking journal in Clinical Medicine, New England Journal of Medicine (Chan et al *NEJM* 2017;377:513-522). The findings of this study affirms that detection of early stage cancers is feasible via screening which led to downstaging of the identified cases ensuing in improved survival. Due to these profound observations, a NPC screening programme is being planned for clinical implementation.

2. **Development of a plasma DNA tissue mapping test (Sun et al *Proc Natl Acad Sci U S A.* 2015;112:E5503-12).** DNA from different tissues or organs bear different DNA methylation profiles. We compared the DNA methylation profiles of a number of tissues and developed an algorithm that allows one to determine the proportional contributions of plasma DNA from various organs. The led to the possibility to use a blood test to assess which tissue or bodily organ is contributing an excess of plasma DNA signifying the development of pathology in the organ. We applied the algorithm to identify the site of origin of cancer. We also determined the tissues that contributed to chromosomal aneuploidies that were detected in plasma. Subsequent to the publication of our study, a new research field had emerged and many more researchers have begun to study methods for "tissue mapping". Consequently, our publication has been cited 65 times just in two years. The citation statistics of this article was the top 1% in clinical medicine and attained the "highly cited" status as documented by the Essential Science Indicators in 2017. There are already a number of applications emerging from this work (see item 4 below).

3. **Resolved a longstanding controversy regarding the lengths of ctDNA.** In the literature, some researchers reported that cancer is associated with shortening of plasma DNA while others reported the association with lengthening of plasma DNA. We compared the lengths of plasma DNA from genomic regions showing copy number gains against regions with copy number losses in plasma of patients with hepatocellular carcinoma. We observed that plasma DNA from regions with copy number gains (i.e. enriched with ctDNA) are shorter than plasma DNA from regions with copy number losses (i.e. depleted of ctDNA). These data suggest that ctDNA molecules are shorter than non-tumour derived DNA. On the other hand, when one studies the overall (tumour and non-tumour) size profile of plasma DNA, there are size variations that correlated with the tumour DNA fraction in the sample. If the sample contained a high abundance of ctDNA, the overall plasma DNA size profile would

be shortened when compared with persons without cancer. However, the overall size profile would be lengthened when the sample contained a low amount of ctDNA. The longer DNA molecules might be derived from cellular damage of the surrounding non-cancerous tissues. These data (Jiang et al *Proc Natl Acad Sci USA* 112: E1317-25) have therefore reconciled a longstanding unsolved question underlying the discrepancies observed with respect to ctDNA length.

4. **Elucidated the biology of circulating DNA.** We believe that a better understanding of the biology of circulating DNA would allow one to be better equipped at developing diagnostics for cancer assessment. Extending from the tissue mapping approach described above, we asked a biological question of whether erythroid DNA is detectable in human plasma. Human erythrocytes are devoid of nuclei. But immature erythrocytes do possess nucleic. During maturation, enucleation would take place. We asked if some of such DNA may be present in plasma. We identified a number of DNA methylation markers that are specific to erythroid DNA, To our surprise, some 20% of plasma DNA molecules are erythroid in origin. This phenomenon was first reported by our group (Lam et al *Clin Chem.* 2017;63:1614-1623) subsequently followed by similar reports from other groups. We further showed that abundance of erythroid DNA in plasma correlated with bone marrow activity with potential value in the assessment of anaemia.
   On the other hand, we studied the nature of urinary DNA (Cheng et al *Clin Biochem* 2017;50:496-501). Using tissue mapping algorithm, we showed that majority of urinary DNA are derived from tissues of the renal tract. Urinary DNA molecules are much shorter than plasma DNA. There is continuous degradation of DNA molecules as it passes from the renal pelvis to the urinary bladder. Such baseline information regarding urinary DNA is important because it forms the basis for our further exploration of identifying bladder cancer derived DNA signatures in urine (work in progress).

5. **Strong foundation to exploit non-invasive cancer diagnostics.** During the course of this research project, the team was able to elucidate a number of fundamental biological features of circulating DNA and ctDNA (as described above) as well as establish a number of foundational methods to exploit the diagnostic potential of ctDNA analysis. Such methods include the identification of cancer-derived chromosomal copy number aberrations (Chan et al *Clin Chem* 59: 211- 224), single nucleotide variants, genomewide methylomic changes (Chan et al *Proc Natl Acad Sci USA* 110: 18761-8), and ctDNA size based alterations (Jiang et al *Proc Natl Acad Sci USA* 112: E1317-25). This all-rounded and efficient approach that the team undertook enabled the team to secure its intellectual property (IP) position. Many of the ensuing patent applications filed by the team are of the earliest priority dates in the field. Hence, many of these patents are of seminal importance. A number of these applications have proceeded to grant.

6. **Technology transfer.** With the strong IP position and promising technologies, Professor Dennis Lo, Rossa Chiu and Allen Chan was able to gather interests from investors to establish a company named Cirina (derived from **Cir**culati**n**g **N**ucleic **A**cids). Cirina was set up as a global company with laboratories based at the Hong Kong Science and Technology Parks as well as South San Francisco. Cirina's goal was to develop and market blood tests for early cancer detection (https://www.genomeweb.com/molecular-diagnostics/startup-cirina-raises-12m-develop-non invasive-tests-early-cancer-screening). Within 2 years of the founding of Cirina, a merger with GRAIL was completed in May 2017 (https://globenewswire.com/news-release/2017/05/31/1003871/0/en/GRAIL-and-Cirina-Co mbine-to-Create-Global-Company-Focused-on-Early-Detection-of-Cancer.html). GRAIL is based in Menlo Park and is also dedicated to the mission of developing blood tests for early

cancer detection. GRAIL has attracted > US$1B in investments. The merger has placed GRAIL and Cirina in a strong position to deliver the mutual goal of curing cancer through early detection. The merger also represented a vote of confidence on technologies developed in Hong Kong.

6.2 What was the added value of the TRS funding, rather than standard project grant funding?
(For example, could this work have been achieved with other funding scheme, such as the General Research Fund or Collaborative Research Fund? If not, why?)

The TRS funding was instrumental in catapulting the project team into its current world leading position in ctDNA research. Before the TRS project, many teams around the world were involved with ctDNA research. While the CUHK team had been studying ctDNA, we were not as internationally well known for our research on cancer diagnostics. Since having been awarded the TRS funding in 2011, the team was able to recruit staff and students to reach a critical mass that could pursue research goals competitively. The bioinformatics team has expanded 3 times in size during the project. The funding duration of 5 years also allowed us to focus on the research without the need for repeated application for grants of smaller funding amount. The funding amount also allowed the team to plan studies that required more extensive resources (for example, the NPC community screening study). Many of our research goals could be addressed in parallel that further enhanced our efficiency. Because of our high efficiency, many patent applications were filed in a timely manner, resulting in our current strong IP position. Our strong IP position was one of the prime reasons of our team's attractiveness to investors who financed our affiliated companies. In short, all of the above would not have been possible if not for the TRS funding.

6.3 If the project has not met its original objectives, why?

All of the original objectives have been met.

6.4 (a) Peer-reviewed journal publication(s) arising directly from this project:
(Please attach a copy of the 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. Please mark the symbol "#" next to the publications involving inter-institutional collaborations)

The Latest Status of Publications				Author(s) *(denote the corresponding author with an asterisk*)*	Title and journal/book *(with the volume, pages and other necessary publishing details specified)*	Submitted to the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknow-ledged the support of RGC *(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)*						

The Latest Status of Publications				Author(s) *(denote the corresponding author with an asterisk*)*	Title and journal/book *(with the volume, pages and other necessary publishing details specified)*	Submitted to the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknow-ledged the support of RGC *(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)*						
2013				Chan KCA, Jiang P, Zheng YWL, Liao GJW, Sun H, Wong J, Siu SSN, Chan WC, Chan SL, Chan ATC, Lai PBS, Chiu RWK, Lo YMD*	Cancer genome scanning of plasma: detection of tumor-associated copy number aberrations, single-nucleotide variants and tumoral heterogeneity by massively parallel sequencing. Clin Chem 59: 211- 224	2012	No	Yes	No
2013				Chan KCA, Jiang P, Chan CWM, Sun K, Wong J, Hui EP, Chan SL, Chan WC, Hui DSC, Ng SSM, Chan HLY, Ma BBY, Chan ATC, Lai PBS, Sun H, Chiu RWK, Lo YMD*	Noninvasive detection of cancer-associated genome-wide hypomethylation and copy number aberrations by plasma DNA bisulfite sequencing. Proc Natl Acad Sci USA 110: 18761-8.	2013	No	Yes	No
2014				Chan RWY, Jiang P, Peng X, Tam LS, Liao GJ, Li EK, Wong PC, Sun H, Chan KCA, Chiu RWK, Lo YMD*	Plasma DNA aberrations in systemic lupus erythematosus revealed by genomic and methylomic sequencing. Proc Natl Acad Sci USA 111: E5302-11.	2015	No	Yes	No

The Latest Status of Publications				Author(s) *(denote the corresponding author with an asterisk*)*	Title and journal/book *(with the volume, pages and other necessary publishing details specified)*	Submitted to the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknow-ledged the support of RGC *(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)*						
2015				Jiang P, Chan CWM, Chan KC, Cheng SH, Wong J, Wong VW, Wong GL, Chan SL, Mok TSK, Chan HLY, Lai PBS, Chiu RWK, Lo YMD*	Lengthening and shortening of plasma DNA in hepatocellular carcinoma patients. Proc Natl Acad Sci USA 112: E1317-25.	2015	No	Yes	No
#2015				Sun K, Jiang P, Chan KCA, Wong J, Cheng YK, Liang RH, Chan WK, Ma ES, Chan SL, Cheng SH, Chan RW, Tong YK, Ng SS, Wong RS, Hui DS, Leung TN, Leung TY, Lai PBS, Chiu RWK, Lo YMD*	Plasma DNA tissue mapping by genome-wide methylation sequencing for noninvasive prenatal, cancer, and transplantation assessments. Proc Natl Acad Sci U S A. 2015;112:E5503-12.	2017	Yes	Yes	No
2016				Jiang P, Lo, YMD*	The Long and Short of Circulating Cell-Free DNA and the Ins and Outs of Molecular Diagnostics. Trends Genet. 2016;32:360-71.	2017	Yes	Yes	No
2016				Lo YMD*, Lam WKJ	Tracing the tissue of origin of plasma DNA-feasibility and implications. Ann N Y Acad Sci. 2016;1376:14-7.	2017	Yes	Yes	No

The Latest Status of Publications				Author(s) *(denote the corresponding author with an asterisk*)*	Title and journal/book *(with the volume, pages and other necessary publishing details specified)*	Submitted to the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknow-ledged the support of RGC *(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				Cheng THT, Jiang P, Tam JCW, Sun X, Lee WS, Yu SCY, Teoh JYC, Chiu PKF, Ng CF, Chow KM, Szeto CC, Chan KCA, Chiu RWK, Lo YMD*	Genomewide bisulfite sequencing reveals the origin and time-dependent fragmentation of urinary cfDNA. Clin Biochem. 2017;50:496-501.	2017	Yes	Yes	No
2017				Lam WKJ, Gai W, Sun K, Wong RSM, Chan RWY, Jiang P, Chan NPH, Hui WWI, Chan AWH, Szeto CC, Ng SC, Law MF, Chan KCA, Chiu RWK, Lo YMD*	DNA of Erythroid Origin Is Present in Human Plasma and Informs the Types of Anemia. Clin Chem. 2017;63:1614-1623.	2017	Yes	Yes	No
2017				Chan KCA, Woo JKS, King A, Zee BCY, Lam WKJ, Chan SL, Chu SWI, Mak C, Tse IOL, Leung SYM, Chan G, Hui EP, Ma BBY, Chiu RWK, Leung SF, van Hasselt AC, Chan ATC, Lo YMD*	Analysis of Plasma Epstein-Barr Virus DNA to Screen for Nasopharyngeal Cancer. N Engl J Med. 2017;377:513-522.	2017	Yes	Yes	No

(b) Recognised international conference(s) in which paper(s) related to this project was/were delivered:
(Please attach a copy of each conference abstract)

Month/Year/ Place	Title	Conference name	Submitted to the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknowledged the support of the RGC *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
April/2013/ Singapore	Non-invasive detection of somatic mitochondrial mutations in plasma by massively parallel sequencing in cancer patients.	Joint Conference of HGM 2013 and 21st International Congress of Genetics	2013	No	Yes	No
April/2013/ Seattle	Reconfigurable Acceleration of Short Read Mapping.	IEEE 21st Annual International Symposium on Field-Programmable Custom Computing Machines	2014	No	Yes	No
August/2013/ Hong Kong	Cancer genome mapping in the circulation by massively parallel sequencing – a potential method of non-invasive liquid biopsy for detecting cancers	Croucher Summer course on Cancer Biology	2014	No	Yes	No
September/2013/ Suzhou	Methy-Pipe2: Comprehensive bioinformatics pipeline for genomewide methylation analysis	Cold Spring Harbour Asia Conference: Frontiers in bioinformatics and computational biology	2014	No	Yes	No

(c) RGC funding should have been acknowledged in all publication(s)/conference papers listed in (a) and (b) above. If no acknowledgement has been made in any of the publications/ papers, please indicate and provide explanations.

UGC funding has been acknowledged in all the publications.

6.5 To what extent this project has strengthened inter-institutional collaborations and other partnerships?

Sun et al (*Proc Natl Acad Sci U S A*. 2015;112:E5503-12), the study on plasma DNA tissue mapping, involved collaboration with haematologists, pathologists and obstetricians from the Hong Kong Sanatorium & Hospital.

This project mainly allowed the team to forge strong collaborative relationships with a wide-ranging profile of medical specialists. For example, most of our studies involved deep collaborations between pathologists, oncologists, bioinformaticians and hepatologists. However, the studies on systemic lupus erythematosus also brought the team to work in collaboration with rheumatologists and immunologists. The study on erythroid DNA brought the team in contact with gastroenterologists and haematologists. Our investigations on urinary DNA led to close collaborations with renal physicians. The NPC population screening also entailed collaborations with epidemiologists and biostatataticians.

As mentioned earlier, the project also brought opportunities to the project team for working more closely with the commercial sector, the biotechnology industry and the Hong Kong Science and Technology Parks.

6.6 Research students trained (registration/awards):

Name	Degree registered for	Date of registration	Date of thesis submission/ graduation
LEE Ka Man Carmen	MPhil	2011	2013
SUN Kun	PhD	2011	2014
GUO Mengbiao	MPhil	2012	2014
PENG Xianlu	PhD	2012	2015
JI Lu	PhD	2012	2015

After graduation, Carmen Lee became a product specialist at Roche Molecular Diagnostics in Hong Kong. More recently, she joined the marketing team at GRAIL. Kun Sun has recently become a Research Assistant Professor at CUHK and continues to serve the team on molecular diagnostics research. Xianlu Peng is now pursuing her postdoctoral fellowship at the Lineberger Comprehensive Cancer Center, University of North Carolina. Lu Ji is now a postdoctoral fellow in the team and has developed a number of novel bioinformatics approaches to circulating nucleic acid analysis.

6.7 Specific products (e.g. software or netware, instruments or equipment developed):

The following tests, molecular analysis approaches or bioinformatics pipelines have been developed and patents have been filed:
Methods for the non-invasive detection of cancer-associated copy number aberrations
Approaches to detect cancer-derived single nucleotide variants among circulating DNA
Approaches for the non-invasive detection of cancer-associated methylomic changes
Plasma DNA tissue mapping methods
Methods for plasma DNA size analysis for cancer assessment
Assessment of cancer by plasma mitochondrial DNA analysis

6.8 Other education activities and/or training programmes developed:

Seminars organised:

Date	Speaker	Seminar title
20 Aug 2012	Yuan Gao, Associate Professor of Department of Biomedical Engineering, Johns Hopkins University, and Director of the Division of Bioinformatics, Genetics, and Epigenetics, Lieber Institute for Brain Development	Mini exons: small size, big impact
4 Nov 2013	Edison Liu, President and Chief Executive Officer, The Jackson Laboratory	Systems Genomics in Cancer Medicine
20 Dec 2013	Wayne Luk, FREng, Professor of Computer Engineering, Department of Computing, Imperial College London	Accelerating Biomedical Computing

30 Jun 2014	Sung Kim, Department of Computer Science and Engineering, Bioinformatics Institute Interdisciplinary Program in Bioinformatics, Seoul National University	Mining Omics Data Helps Understand Phenotype-Specific Biological Mechanisms
12 Jan 2015	Toshikazu Ushijima, Chief of the Division of Epigenomics, Senior Deputy Director, National Cancer Center Research Institute, Japan	Diagnostic and therapeutic translation of cancer epigenetics
14 Oct 2015	Caroline Dive, Deputy Director & Clinical and Experimental Pharmacology Group Leader, Cancer Research UK Manchester Institute, The University of Manchester	Circulating tumour cells in lung cancer – biomarkers, biology, mouse models and drug development
18 Dec 2015	Wayne Luk, FREng, Professor of Computer Engineering, Department of Computing, Imperial College London	Reconfigurable acceleration of genomic data analysis and compression
5 Apr 2016	Toshikazu Ushijima, Chief of the Division of Epigenomics, Senior Deputy Director, National Cancer Center Research Institute, Japan	Epigenetic and genetic fields for cancerization: towards precision cancer risk diagnosis

Symposium sessions organised:

During the 18th Annual Scientific Symposium of the Hong Kong Cancer Institute, 23 to 24 November, 2013, Hong Kong

09:30-10:30	**Molecular Analysis of Breast Cancer: supported by the Hong Kong Research Grants Council Theme-based Research Scheme (T12-404/11)**	**Tsz Kok YAU** **OncWell Integrated Cancer Centre** **Diana SIU** **Hong Kong Sanatorium & Hospital**
09:30-09:50	*Hereditary Breast and Ovarian Cancer: Experience of The Hong Kong Hereditary Breast Cancer Family Registry and Status in Asia*	**Ava KWONG** University of Hong Kong, HKSAR, China
09:50-10:10	*Cancer Screening: the Potential of Next Generation Sequencing*	**Rossa CHIU** The Chinese University of Hong Kong, HKSAR, China
10:10-10:30	*Pharmacogenetics Analysis in Asian Breast Cancer Patients*	**Soo Chin LEE** National University of Singapore, Singapore

During the 19th Annual Scientific Symposium of the Hong Kong Cancer Institute, Frontiers in early drug development in oncology and internal medicine, 22 to 23 November, 2014, Hong Kong

14:30-15:30	**Translational Session: MD Anderson Sister Institution Symposium (supported by the Hong Kong Research Grants Council Theme-based Research Scheme (T12-404/11))**	**Ka Fai TO** The Chinese University of Hong Kong, HKSAR **Vivian LUI** The University of Hong Kong, HKSAR
14:30-14:50	*Systems Approach to Personalized Molecular Medicine*	**Gordon MILLS** MD Anderson Cancer Centre, Houston, USA
14:50-15:10	*A Generic Cancer Test: Genomic Analysis of Plasma DNA*	**Allen CHAN** The Chinese University of Hong Kong, HKSAR
15:10-15:30	*Phase I Clinical Trials in Oncology at SYSUCC, and Molecular Pre-screening Program*	**Li ZHANG** Sun Yat Sen University Cancer Centre, Guangzhou, China

During the 20th Annual Scientific Symposium of the Hong Kong Cancer Institute, Breast cancer conference, 7 to 8 November, 2015, Hong Kong

09:30-10:55	**Translational & Diagnostic Research in Breast Cancer: Supported by the Hong Kong Research Grants Council Theme-based Research Scheme (T12-404/11)**	**Rossa CHIU** The Chinese University of Hong Kong, HKSAR, China **Wing Cheong CHAN** North District Hospital, HKSAR, China
09:30-10:05	*The Relevance of Immune Aspect in Breast Cancer*	**Paul MAINWARING** ICON Cancer Care, Brisbane, Australia
10:05-10:25	*Triple Negative Breast Cancer - Correlation with Other Markers to Predict Outcomes*	**Gary TSE** Prince of Wales Hospital, HKSAR, China
10:25-10:45	*Distinct Clinicopathological Features and Potential Etiology of Emerging Young Female Breast Cancer Patients in East Asia*	**Ching-Hung LIN** National Taiwan University, Taiwan
10:45-10:55	Panel Discussion	

6.9 Please highlight any deliverables indicated in the project implementation timetable endorsed by the RGC which have not been covered or achieved as per sections 6.1 to 6.8 above, and explain/ elaborate.

All deliverables indicated in the project implementation timetable have been achieved.

Project Management

6.10 Please elaborate how the PC has played his/her role in coordinating and managing the project.

The PC led the project team, determined the project directions and set the priorities throughout the grant duration. The PC made decisions for the timely acquisition of new technologies which enabled the team to weather through the competitive landscape within the research field. The PC was active in disseminating the research findings by participating in many conferences and symposia around the world (see section 7.2 below). The PC supervised a proportion of the postgraduate students and held regular data review and progress meetings. The PC identified and nurtured talents. For example, Prof Peiyong Jiang was formerly Prof Hao Sun's PhD student. Prof Jiang's academic potential was realised and the PC recommended his pursuance of an academic career. Prof Jiang is now an Assistance Professor at the Department of Chemical Pathology, CUHK and the lead bioinformatician for this project. The PC was actively involved in the conceptualisation of new methodologies, patent filing and patent prosecution that has led to the team's strong IP position.

7. Awards and Recognition

7.1 Have any research grants been awarded that are directly attributable to the results obtained from this project?

Yes, the Research Grants Council Theme-based Research Scheme project (T12-401/16-W), titled, Centre for Research into Circulating Fetal Nucleic Acids, that started in December 2016 was a project funded with HKD44.4M for extending the work of this TRS project.

In addition, the merged entity of GRAIL/Cirina has entered into a contract research agreement with the project team to provide funding for translational research in cancer diagnostics and to facilitate cross-training of scientists between the company and project team.

7.2 Have any project team members participated as invited speakers in or organisers of international conferences as a result of this project?

Yes, the project team participated in a total of 36 international meetings as invited speakers.

1. Chiu RWK. "Noninvasive delivery of the cancer genome by next-generation sequencing of plasma DNA", The 17th Annual Scientific Symposium of the Hong Kong Cancer Institute: Clinical Application of Biomarkers in Cancer Therapy. 25 November 2012, Hong Kong.
2. Lo YMD. "Plasma DNA sequencing for prenatal and cancer testing", Annual General Meeting and Scientific Lecture, The Association of Clinical Genetics and Genomics of Hong Kong. 11 April 2013, Hong Kong.
3. Chiu RWK. "Cancer Genome Assembly through Plasma DNA Analysis", Royal College of Pathologists of Australasia: Short Course in Medical Genetics and Genetic Pathology June 2013. 17 June 2013, Gold Coast, Australia.
4. Chan KCA. "Circulating Nucleic Acids Markers for Cancer", International Meeting on Cell-free DNA. 20-21 June 2013, Copenhagen, Denmark.
5. Lo YMD. "Detection of genome-wide cancer-associated molecular alterations in plasma using shotgun massively parallel sequencing", 2nd Taiwan Epigenomics Symposium, 5 July 2013, Chiayi, Taiwan.
6. Lo YMD. "Massively parallel sequencing of plasma DNA for non-invasive prenatal diagnosis and cancer detection", BSGM 2013, British Society for Genetic Medicine, Liverpool, United Kingdom, 16-18 September 2013.
7. Lo YMD. "Translating novel molecular diagnostic tests into clinical", Hong Kong Academy of Medicine 20th Anniversary Congress, Hong Kong Academy of Medicine, Hong Kong, China, 8-10 December 2013.
8. Lo YMD. "Extracellular DNA in plasma: from science to clinical applications", Frontiers in Biomedical Research, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China, 13 December 2013.
9. Lo YMD. "Plasma DNA Sequencing for Noninvasive Prenatal Testing and Cancer Detection", Molecular Med TRI-CON2014 (Personalized Diagnostics) organized by Cambridge Healthtech Institute, San Francisco, U.S.A., 13-14 February 2014.
10. Chiu RWK. "Prenatal and cancer diagnostics by plasma nucleic acid analysis", International Conference of Advanced Technologies 2014 jointly organized by Hong Kong Polytechnic University, HKSMDS, Chinese American Association for Clinical Microbiology & Association for Molecular Pathology, Hong Kong, China, 7-9 March 2014. Presented by R Chiu.
11. Lo YMD. "Prenatal diagnosis and cancer detection using plasma DNA: two sides of the

same coin", Distinguished Lecturer Seminar Series organized by German Cancer Research Centre, Heidelberg, Germany, 8-11 Mary 2014.

12. Lo YMD. "Plasma DNA sequencing for non-invasive prenatal testing and cancer detection", Genomics and Stem Cell Based Therapies: Shaping the Future of Personalized Medicine jointly organized by State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Centre, Sun Yat-sen University and Nature, Guangzhou, China, 29-31 May 2014.
13. Lo YMD. "Deciphering the plasma genome: applications to prenatal diagnosis and oncology", IFCC WorldLab Istanbul 2014 organized by Turkish Biochemical Society, Turkey, 22-26 June 2014.
14. Chiu RWK. Plenary lecture: "Non-invasive scanning of the cancer genome using plasma samples collected from cancer patients - detect copy number gains and losses as well as the methylation profile in a genomewide fashion for solid tumours but using plasma DNA", Annual Scientific Meeting of the Human Genetics Society of Australasia, Adelaide, Australia, 5 August, 2014
15. Chiu RWK. "Deciphering the Plasma Genome for Prenatal and Cancer Medicine", Personalised Medicine Symposium: From Science to Clinical Decisions jointly organized by Li Ka Shing Institute of Health Sciences, CUHK and Centre for Personalised Medicine, University of Oxford, 3 November, 2014.
16. Lo YMD. "Plasma DNA tissue mapping: application to nonivasive prenatal testing, cancer detection and transplantation monitoring" in Cell Symposium: Human Genomics on 8-10 November, 2015, organized by Genome Institute of Singapore, Cell Reports and Cell, Singapore
17. Lo YMD. "Plasma DNA as a Treasure Trove for Molecular Diagnostics" in Institute for Human Genetics (IHG) Symposium 2015 on 2 November, 2015, organized by Institute for Human Genetics (IHG) at the University of California San Francisco, USA
18. Chiu RWK. "Hunting for the signatures of cancer by plasma DNA sequencing", Royal College of Pathologists of Australasia, Pathology Update 2016, Melbourne, Australia, 26 February 2016.
19. Chiu RWK. "Plasma nucleic acids: a treasure trove of diagnostic information", Children's Hospital of Philadelphia and University of Pennsylvania, 8 February, 2016.
20. Chiu RWK. "Circulating nucleic acids: a treasure trove of diagnostic information", Institute of Biomedical Sciences, Hong Kong branch Annual meeting, Hong Kong, 30 October, 2015.
21. Chiu RWK. "Liquid biopsy as a total body scan: unravelling the sites of cancer and other pathologies", China Med—the 8th Forum on Laboratory Medicine, Beijing, China, 26 March 2016.
22. Lo YMD. "Plasma DNA as a Treasure Trove for Molecular Diagnostics" in The 27th Annual Meeting of the German Society of Human Genetics on 16-18 March, 2016, organized by German Society of Human Genetics, Lubeck, Germany

23. Lo YMD. “Advancing the Frontier of DNA Testing” in Hospital Authority Convention 2016 on 3-4 May, 2016, organized by Hospital Authority, Hong Kong
24. Lo YMD. “Genome-wide plasma DNA sequencing as a universal approach for cancer detection” in The European Human Genetics Conference (ESHG 2016) on 21-24 May, 2016, organized by European Society of Human Genetics and European Meeting on Psychosocial Aspects of Genetics, Barcelona, Spain
25. Lo YMD. “Liquid Biopsies from Prenatal Diagnosis to Cancer Testing” in MIT Technology Review's EmTech Hong Kong on 7-8 June, 2016, organized by MIT Technology Review and Koelnmesse Pte Ltd, Hong Kong
26. Lo YMD. “Early detection of plasma DNA for cancer screening: What may the future look like?” in The Sondland-Durant Early Detection of Cancer Conference on 21-24 June, 2016, organized by The Knight Cancer Institute at Oregon Health & Science University and Cancer Research UK, Portland Oregon, USA
27. Lo YMD. “Plasma DNA Analysis for Detecting NPC: Biological Insights and Clinical Applications” in Gordon Research Conference: Nasopharyngeal Carcinoma on 26 June - 1 July, 2016, organized by Gordon Research Conferences, Hong Kong
28. Lo YMD. “Unlocking Hidden Diagnostic Information from Plasma DNA” in Gordon Research Conference: Personalized Medicine on 10-15 July, 2016, organized by Gordon Research Conferences, Hong Kong
29. Chiu RWK. “Plasma nucleic acids: a treasure trove of diagnostic information”, Gordon Research Conference in Advanced Health Informatics, Hong Kong, 20 July, 2016.
30. Lo YMD. “Creating a paradigm shift in medical diagnostics using circulating DNA” in Hong Kong College of Health Service Executives (HKCHSE) Annual Conference 2016 on 23 July, 2016, organized by Hong Kong College of Health Service Executives (HKCHSE), Hong Kong
31. Chiu RWK. “Investigations of circulating tumor cells (CTCs), cell free DNA (cfDNA), and microRNA (miRNA) in the diagnosis of cancer”, Annual meeting of the American Association of Clinical Chemistry, Philadelphia, USA, 3 August 2016.
32. Chiu RWK. “Liquid biopsy as a total body scan: unravelling the sites of cancer and other pathologies”, Annual meeting of the American Association of Clinical Chemistry, Philadelphia, USA, 2 August 2016.
33. Chiu RWK. “Update in the current status of liquid biopsy in cancer management”, Molecular Pathology Symposium, Griffith University and MD Anderson Cancer Group, Gold Coast, Australia, 11 September, 2016.
34. Chiu RWK. “Liquid Biopsy as a Total Body Scan: Unravelling the Sites of Cancer and Other Pathologies”, 25th annual Royal Brisbane and Women's Hospital Healthcare Symposium, Brisbane, Australia, 12 to 16 September, 2016.
35. Lo YMD. “Plasma DNA: Driver of a revolution in molecular diagnostics for the clinic” in 4th Joint EFLM - UEMS Congress "Laboratory Medicine at the Clinical Interface" on 21-24 September, 2016, organized by The European Federation of Clinical Chemistry and

Laboratory Medicine (EFLM) and The European Union of Medical Specialists (UEMS), Warsaw, Poland

36. Lo YMD. "Solid tumour, liquid biopsy: can blood samples give us the answers?" in The 41st European Society for Medical Oncology Congress (ESMO 2016) on 7-11 October, 2016, organized by The European Society for Medical Oncology (EMSO), Copenhagen, Denmark

7.3 Have any project team members taken leadership positions in editorial boards, scientific and professional organisations?

Yes, Prof Dennis Lo and Prof Rossa Chiu are both Associate Editors of *Clinical Chemistry*, the No. 1 ranked journal in laboratory medicine. Prof. Lo is also an Associate Editor the Nature Partner Journal, *Genomic Medicine*. Prof Lo is an Editorial Board member of the *Journal of Pathology*, *Philosophical Transactions of the Royal Society B*, *Disease Markers*, *Chimerism*, and *Marrow*. Prof Chiu was an Associate Editor of *Clinical Biochemistry* and is also an Editorial Board member of *Clinical Chemistry and Laboratory Medicine*.

Prof Dennis Lo has been serving as a Council Member of the Research Council as well as the co-chairman of the Grant Review Board of the Food and Health Bureau of Hong Kong since 2012. Prof Rossa Chiu is a Member of the Research Grants Council Biology and Medicine Panel (Joint Research Scheme) since 2012. She is also a member of the Innovation and Technology Fund Research Projects Assessment Panel (Biotechnology), Innovation and Technology Commission, Government of HKSAR, since 2017.

7.4 Any documentary proof of the application of technologies arising directly from this project?

The plasma EBV DNA test for NPC screening is being planned for launch as a clinical service.

7.5 Other awards and recognitions as a result of this project (please specify):

Prof Dennis Lo, Project Coordinator, was elected as a Fellow of the Royal Society in 2011, awarded the AACC-NACB Award for Outstanding Contributions to Clinical Chemistry in a Selected Area of Research for year 2012, National Academy of Clinical Biochemistry, USA; the 2012 Ernesto Illy Trieste Science Prize in Human Health from the TWAS the Academy of Sciences of the Developing World (presented by HU Jintao, President of China); selected as a Foreign Associate of the National Academy of Sciences of USA in 2013; awarded the King Faisal International Prize for Medicine 2014, the International Society for Blood Transfusion Presidential Award 2014, elected as a Fellow of The World Academy of Sciences in 2014, elected as a Founding Fellow of the Academy of Sciences of Hong Kong in 2015, a Thomson-Reuters Citation Laureate in Chemistry in 2016 and the winner of the Future Science Prize in Life Science in 2016.

8. Impacts

8.1 What are the current and expected impacts of the project on the long-term development of Hong Kong (social or economic development, e.g. patent, technology transfer, collaboration with external organisations, etc.)?

We believe that the achievements of this project has already placed Hong Kong based biotechnology onto the global map. Through this project, the project team has secured a strong IP position. We have filed patent applications covering a diverse array of methods to analyse ctDNA. Many of these filings have achieved the earliest priority dates in the field and are therefore likely to become foundational patents upon which additional technologies are built. A number of the earlier filings have proceeded to grant in a number of jurisdictions affirming the successful strategies adopted by the team. This strong IP position is further recognised and acknowledged by the commercial sector resulting in the successful financing of the startup company, Cirina, founded by Professors Dennis Lo, Rossa Chiu and Allen Chan. The value of Cirina and its mission to cure cancer by achieving early detection resonated well with GRAIL which culminated in the merger between the two companies. This global union of the two companies with IP transfer from CUHK is a testimony to the value and innovativeness of the team's research which has been developed based in Hong Kong. Going forward, the tests and approaches developed by the project team will be commercially launched. It is hope that widespread adoption could be achieved resulting in reduction in cancer-related mortality and morbidity.

8.2 Others (please specify):

Nil

9. Sustainability of the Project

9.1 Whether there are new ideas evolved directly from this project?

Yes, there have been many novel ideas developed during this project as evident by the team's patent portfolio. Many of the patent applications have been granted in a number of jurisdictions around the world. The successful granting of these applications is an indication of the novelty of the concepts developed during the course of the project. The novel concepts developed during the course of the project and covered by patent applications are listed in section 6.7 above.

9.2 Whether there are new projects evolved directly from this project?

Yes, in order to study the biology of circulating DNA, during the course of this project, we studied the molecular characteristics of DNA in plasma of patients with systemic lupus erythematosus (SLE) (Chan et al *Proc Natl Acad Sci USA* 111: E5302-11). It is known that anti-dsDNA antibodies are present in the circulation of SLE patients. We wondered if these antibodies would bind circulating DNA and perturb its profile. Indeed, we found that anti-dsDNA antibodies do bind circulating DNA. There is preferential binding to short DNA fragments and DNA that is hypomethylated. Consequently, the plasma DNA profile of SLE patients with active disease is characterised by shorter size distributions, hypomethylation and perturbed chromosomal distributions. Due to these aberrant profiles, we were able to distinguish the plasma DNA profiles of patients with active SLE exacerbations against those with inactive disease. Thus, a new approach for the clinical assessment of SLE was developed and we are actively studying the clinical value of these approaches.

By extending the tissue mapping approach, we have succeeded in proving the existence of erythroid DNA in plasma (Lam et al *Clin Chem* 2017;63:1614-1623). Coincidentally, the abundance of erythroid-derived DNA in plasma reflects bone marrow activity. This may become a novel approach for the clinical assessment of anaemia. For example, we showed

that the amount of plasma erythroid DNA was elevated in patients with ineffective erythropoiesis but not in patients with unresponsive aplastic anaemia.

In addition, we have now extended our studies onto urinary DNA (Cheng et al *Clin Biochem* 2017;50:496-501). We have gained a wealth of information regarding its molecular characteristics and are currently exploring a number of clinical applications based on urinary DNA analysis.

9.3 Whether there are new collaborations developed directly from this project?

Yes, the studies on SLE led to our collaboration with rheumatologists and immunologists for the first time. Our studies on erythroid DNA brought us to work closely with haematologists and gastroenterologists The urinary DNA studies led to our working with renal physicians. All of these are newly developed collaborative relationships.

9.4 Please give details on how much money and from which sources has been obtained/requested for the specific purpose of continuing the work started under this project.

HKD44.4M has been granted under the Theme based Research Scheme project (T12-401/16-W), titled, Centre for Research into Circulating Fetal Nucleic Acids, for continuing the research of this TRS project.

In addition, the merged entity of GRAIL/Cirina has entered into a contract research agreement with the project team to provide funding for translational research in cancer diagnostics and to facilitate cross-training of scientists between the company and project team. Due to the confidentiality clauses in the agreement, the financial terms of the funding support could not be disclosed.

Project Title: Massively Parallel Sequencing of Plasma Nucleic Acids for the Molecular Diagnostics of Cancers
Project Coordinator: Prof Dennis Yuk-ming Lo (CUHK)

Summary

Cancer is the top killer in Hong Kong and many parts of the world. The lack of effective tools for the timely detection and dynamic monitoring of cancer has hindered efforts in combatting cancer. The Project Team is a world-leading group in the biology and diagnostic applications of plasma nucleic acids. The advent of massively parallel DNA sequencing has created a paradigm shift in genomics research. The group is among the first to demonstrate the use of massively parallel sequencing as a diagnostic tool. The group has pioneered a number of novel approaches for applying massively parallel sequencing for the detection and analysis of plasma nucleic acids at an unprecedented level of sensitivity, resolution and comprehensiveness. In this project, the group proposed to lay the conceptual and technological foundation for applying massively parallel sequencing of plasma nucleic acids as a detection and monitoring tool for cancer. The team aimed to develop technologies that would allow a non-invasive genome-wide scan of cancer-associated genetic, transcriptomic and epigenomic alterations in plasma. Such an approach would allow the development of generic cancer detection tests with broad population coverage and unlike the approaches available to date where only a subset of cancer-related molecular alterations are targeted at each instance. Coupled with the proven analytical power of massively parallel sequencing, such developments are expected to enable the timely detection, real-time monitoring and accurate prognostication of cancer.

During the project, the team has developed a powerful suite of technologies for detecting cancer-related genomic signatures in blood plasma. Work by the project team had been selected as one of the top ten breakthroughs in 2015 by the influential technology magazine, MIT Technology Review (https://www.technologyreview.com/s/534991/liquid-biopsy/). In 2017, the project team has demonstrated in a highly publicized clinical study published in the New England Journal of Medicine that circulating DNA testing would greatly increase the proportion of early nasopharyngeal cancer (NPC) cases detected, resulting in improved progression-free survival (http://www.nejm.org/doi/full/10.1056/NEJMoa1701717). When launched, the project team believes that this test could potentially reduce mortality due to NPC in south China by 50%. The project team has created a valuable intellectual property (IP) portfolio covering these technologies. Members of the project team has set up a startup company, Cirina, in the Hong Kong Science Park. Cirina has licensed the IP portfolio from The Chinese University of Hong Kong (CUHK). In June 2017, Cirina merged with Grail in the USA. This combined company is the most highly valued (over US$ 2 billion) on in the liquid biopsy space worldwide. This project is thus one notable example that has brought Hong Kong-based biotechnology into the global arena.